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MEDICAL PARASITOLOGY IN A CHANGING WORLD. WHAT OF THE FUTURE?*

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The presidential addresses of three of the last four retiring presidents of this Society have embraced the field of medical parasitology. Dr. Stoll discussed this wormy world; Dr. Faust gave his reflections of a medical parasitologist; and Dr. Cameron recounted the record of Sir Wm. Osler as a parasitologist. This address perforce continues the theme since the speaker's present interest lies almost wholly within this field. Perhaps those members with other interests will bear with me on the strength of the hope that they may later have better representation in this annual event.

In the program this morning, we have marked the 25th anniversary of the founding of the American Society of Parasitologists. This might be considered a suitable time for taking an inventory of accomplishments of the Society or accomplishments in the general field of parasitology. Rather, I should like to review the possibilities for the future and to visualize the needs and opportunities which lie before us in this particular branch of the science.

EXPANDING HORIZONS

As a result of World War II, the United States has been forced into a position of world leadership. This position entails certain responsibilities. A considerable number of countries are greatly dependent upon us for support. We in turn, more than ever before, are dependent on certain parts of the world for assistance in our national defense scheme and for our economic welfare. These relationships have been indubitably established. It seems probable that present policies with regard to international assistance will be continued for sometime to come. You or I, as individuals, may not concur in the political philosophy which governs these cooperative schemes. At least for the time being we must accept them as representing the best judgment of our political minds and as one possible solution for the world's present ills.

Fifty years ago world health was of little concern to us except from humanitarian and quarantine standpoints. Now, low health standards with loss of man power and lower commodity production, mean additional shrinkage in trade balances. Together with lack of capital investment, they may spell failure to develop natural

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resources in backward areas, a development which is sorely needed to bolster world economy.

Fortune Magazine (1), which reflects the thinking of big business, and which is usually critical of our international assistance programs, has this to say about the Point Four scheme:

> "Alongside the topless towers of Point Four industrial dreaming, public health and rural sanitation seem a humdrum issue, something to be left to Rockefellers and missionaries. Actually they deserve first priority for economic as well as humane reasons. The short life expectancy of an Indian (twenty-seven years) or a Persian is itself their No. 1 national economic handicap and worst form of 'underemployment,' since it means—to put it coldly—that the average child born is destined to spend too much of its life as a drain in proportion to its years of productivity. To urge birth control is only to beg this question; for able-bodied adults are always a potential economic asset. Colonial public-health programs made possible India's and Indonesia's enormous population growth, which is troublesome but not basic, for that growth measures the first stage of economic development."

It is heartening that a periodical of this type has taken such an understanding view of the importance of public health in these assistance programs. Under the tentative allocations, 20.2 per cent of Point Four funds will be employed for extending aid in this field and in furnishing individuals with the "know-how" for work in backward areas where their services are badly needed. This has been done to some extent under the ECA program but will be carried out in a larger measure under Point Four. In addition, the amount of $20,000,000 recently allocated by UNO for the habilitation of backward areas will in part go to WHO for public health and preventive medicine projects.

THE TOLL OF PARASITIC DISEASES

Health standards in backward areas are immeasurably influenced by the prevalence of parasitic diseases. Since these diseases are chronic in nature and are seldom an immediate cause of death, they are of particular economic importance. A dead person requires no food but an individual chronically ill with malaria or sleeping sickness still requires sustenance. He may not be able to produce his share and thus becomes an economic burden on his family and his community. If his case is multiplied by tens of thousands or hundreds of thousands, one arrives at the sum total of the cost of parasitic diseases in a given area. It is unfortunate that so few factual data are available to measure the cost of parasitic diseases. If the figures were known, they would be staggering.

In Egypt, it is estimated that schistosomiasis is costing the country as a whole approximately 20 million pounds per year. The productivity of the population is decreased by 33 per cent. All of the heavy labor in Egypt is supplied from Upper Egypt, where the disease has a very low incidence. Over a period of years, 22 percent of Army recruits from Lower Egypt have been rejected for physical defects, whereas only 3 per cent of those from Upper Egypt have failed to pass the physical examinations. This wide difference is believed to be due almost entirely to the high schistosome infection rate in Lower Egypt.

In a representative month on a sisal estate in Tanga Province, Tanganyika, 40.5 per cent of the total employees applied for treatment at the company hospital (2). Of the total force, 8 per cent had malaria, 9 per cent hookworm disease, 3 per cent tropical ulcer, 2.5 per cent bronchitis and pneumonia, and 18 per cent other

diseases, of which the dysenteries and schistosomiasis constituted the major portion. Therefore, parasitic diseases were responsible for decreased productivity in approximately 30 per cent of the total labor force and were the cause of about 75 per cent of the morbidity.

Malaria is one of the most debilitating of the chronic diseases. Because it is largely a disease of rural areas, it is a major factor in limiting agricultural output as well as a deterrent to the development of fertile and potentially productive regions. It is impossible to give even approximate figures for malaria mortality and morbidity in the world, for in the very regions where malaria takes its heaviest toll, no registration of cases is available. In India in 1944, 4,352,143 deaths from "fever" were registered, of which 1,830,437 were indicated as caused by malaria. In 1945 in Ceylon, the disease was responsible for 8,521 deaths, while more than 2,500,000 malaria cases were treated in registered hospitals and dispensaries. Sinton (3) has calculated that losses from malaria in India amount to $320,000,000 per year. Wright (4) has estimated that the post-war UNRRA malaria control campaign in Greece resulted in a saving of 30 million man-days. Balfour (5) has cited data from a report from a rubber estate in the Federated Malay States to show the economic benefits to be derived from the control of malaria. The comparative figures for the year 1911, prior to the application of control measures, and 1923 when control had become established are reproduced in Table 1. By 1923, this particular plantation had become one of the cheapest producers of rubber in the Federated Malay States. This record is all the more significant when it is considered that measures for the control of malaria at that time were far less efficient than those available today.

These figures are mere scraps of information. Cost accounting has had little part in the concept of disease. Large corporations operating in the tropics have been in a position to evaluate the dollar losses from parasitic and other tropical diseases but they do not have this information. At best, they can only say that labor efficiency has increased as disease control has been achieved.

TABLE 1.—*The economic benefits from the control of malaria on a rubber estate in the Federated Malay States. Comparative figures for 1911, prior to application of control measures, and 1923 when control had been achieved*

	1911	1923
Average acres cultivated	1,632	2,650
Average labor force	870*	450
F.O.B. cost of rubber	$1.09	$0.1864
Total yield	83,000 lbs.	778,000 lbs.
Total operating cost	$240,215.38	$145,018.44
Cost of medical care	$12,444	$6,208.67
Amount spent on preventive medicine programs	Nil	$9,531.20
Number of deaths	202	2
Death rate per thousand	232	3
Staff (Europeans)	7	4
Hospital admissions for the year	1,084	275
Total loss of labor (man days)	862	186

* Not including an unrecorded number of Chinese laborers.

MEDICAL PARASITOLOGY AND INTERNAL ECONOMY

If we look behind the facade of assistance programs to backward areas, we may find more substantial values than are apparent on the surface. Between 1936 and 1940, the per capita value of U. S. exports to countries of Northwestern Europe, the British Isles, Australia and New Zealand was $5.80; to Southern Europe, Northern

Asia, South Africa and parts of South America $1.25; and to the remainder of the world, comprising the undeveloped areas, only $0.70. These latter areas have barely enough food to sustain life, a per capita income of only $41.00 per annum, a life expectancy of only 30 years, and only 17 physicians per 100,000 persons. They comprise parts of the globe where parasitic diseases are most highly endemic and most severe in their clinical manifestations. If health conditions are improved and if these and other diseases can be put under control, we may expect an improved economy, greater production and an expanded trade with these countries which are rich in raw materials which this country lacks and acutely needs. It would seem that our unfavorable trade balance could be corrected most rationally through improved commercial relations with underdeveloped areas rather than with countries of Northwestern Europe which compete for the most part with our manufactured products. Puerto Rico may be cited as an example in support of this thesis. The record shows that, as health conditions have improved and the national income has risen, her imports from the United States have increased proportionally during recent years. On a per capita basis, the island is the best overseas market for U. S. products; in amount of goods bought, irrespective of population, she is our fifth best customer in Latin America and thirteenth in the world.

We already have a heavy stake in these tropical areas. In the calendar year 1949, 59.08 per cent of our total imports of $6,598,058,377 came from tropical countries. These imports consisted of food products, such as coffee, cane sugar, cacao, edible nuts, palm oils, tea, bananas, and vegetable oils. They included such highly strategic materials as rubber, tin, bauxite, manganese, chrome, and uranium, which come almost exclusively from tropical areas. In addition, we imported huge quantities of copper, lead, zinc, nickel and nickel alloys, fertilizers, crude industrial chemicals, and petroleum and petroleum products.

This part of the world will be of increasing importance to us as time goes on. Our domestic reserves of many raw materials are running dangerously low (6). For lead and zinc, American industry may become increasingly dependent on Mexico, Peru, and Chile. Prospective depletion of the high-grade iron ore of the Masaba Range has already forced steel companies to seek far afield for future requirements. Six American steel companies in cooperation with the Hollinger North Shore Exploration Co., Ltd. are developing deposits in Labrador. Bethlehem Steel and United States Steel have fabulously rich ore concessions in Venezuela while Republic Steel will soon begin shipping ore from its holdings in Liberia. Other than the Labrador deposits, the best prospects for meeting our iron ore requirements lie in the tropics. In addition to the developments already begun, explorations are being made in Brazil and the Dominican Republic. The far flung quest for this basic material to fill requirements after the depletion of domestic reserves is illustrative of the situation which will confront us in the not too distant future in connection with other strategic materials.

It is an axiomatic fact that any disease or condition which affects the health or well being of the worker in tropical areas must affect the supply and cost of commodities which are imported from these areas. New enterprises will be faced with the necessity of controlling malaria, hookworm disease, filariasis and other tropical diseases. Fortunately, American concerns have been acutely aware of this necessity in the past. The achievements of such a concern as the United Fruit Company in

marketing bananas at a price and in a condition which would attract widespread consumer demand and at the same time compete with domestic food products could never have been attained without a medical department noted for its conquests of these and other tropical diseases. The Firestone Tire and Rubber Company and the Standard Oil Company of New Jersey and its affiliates find it necessary to carry on extensive preventive medicine programs in tropical areas in which they operate. Other American companies have equally good records of accomplishment in this regard.

With expansion of American interests in tropical areas, an increasing demand may be expected for more effective treatments and better control measures for parasitic diseases. With such demand, there should develop more opportunities for research in the field of medical parasitology. Germany was for long years one of the leaders in this field, especially in chemotherapy. After the loss of her tropical colonies following World War I, she still carried on such research with unabated zeal, partly because she probably expected eventually to reclaim these colonies but partly also because many of her raw materials came from tropical climes. Her premier position in this field was of immense propaganda value. Scientists from all over the world flocked to her research laboratories and carried back to their home lands German textbooks and drugs and pharmaceuticals. In fact, for the space of half a century prior to World War II, Germany dominated world markets in drugs for the treatment of tropical diseases. More recently, the United States has assumed some of the leadership in this field. For instance, exports of medicines and pharmaceuticals to Latin American countries increased from $18,000,000 in 1942 to over $88,400,000 in 1949 (7). While absence of European supplies had for a time considerable to do with the dominance of our exports to these countries, the increase is due in part at least to research which has provided new and better products.

During World War II, we should have been in a sorry plight without drugs and insecticides developed as a result of research in Europe. From the standpoint of our own security, it is questionable whether we should continue to depend on foreign sources for research advances in this field.

MEDICAL PARASITOLOGY AND WORLD FOOD SUPPLY

Sir John Orr (8), former Director General of the Food and Agriculture Organization of the United Nations, has estimated that world food production will have to be more than doubled within the next 25 years to prevent calamities. This prediction is both interesting and prophetic in view of the exceptionally rapid increase in the world's population.

During the first decade of this century the mean rate of increase of population in the countries which had a regular census was 1.159 per cent per annum (9). At this rate the numbers would be doubled in a little more than 60 years. If this had been the average rate of increase in the past, the whole of the present population of the world would be descended from one couple living near the end of the first century A.D. If it could be maintained in the future, then in another thousand years the earth would have about 250 millions of millions (25×10^{13}) of human inhabitants, i.e., more than one to every square yard of land. Right now the world's population is nearly 200 million greater than it was before World War II. It is as if another

North America had been added to the consuming population of the globe while the war was being fought (10).

The limiting factor to the growth of population is that of food supply. Outside of entirely icebound areas, the total available land on the globe is about 56 million square miles. Of this, it has been estimated that approximately 30 per cent, or 17 million square miles, is cultivatable (11). Approximately, one-fourth of this cultivatable land lies in the tropics and for the most part is very sparsely settled. Thus this land offers the chief possibilities for any considerable extension in world food production.

The alternatives of this lie in settlement in the dry lands or extension of agriculture to the polar lands. Exploitation of arid or semiarid regions entails a heavy capital investment and can only be profitable when high-value crops are produced. It is even quite likely that the forward surge into the dry realms has gone further than is wise under present conditions (12). The polar lands offer few inducements for agricultural development. The general low temperatures, short growing season, and lack of transportation facilities to far distant markets preclude any great extension of food production in these areas.

In addition to a year around growing season, the tropics offer a supply of cheap labor, which can be maintained at an efficient level provided attention is given to sanitation, medical care, nutrition, and adequate housing. The present Director General of FAO, Mr. Norris E. Dodd, believes that Africa offers the greatest prospects in agricultural development (13). However, on that continent today there are 4½ million square miles of fertile land lying idle and uninhabitable because of sleeping sickness. Here, as elsewhere in the tropics, any coordinated effort to increase food production and agricultural output must have as its first consideration the control of parasitic and other tropical diseases.

In spite of the over-all questionable economy of extension of agriculture into the dry lands, many such projects are planned in various parts of the world. It is the invariable result that irrigation projects in endemic areas of malaria and schistosomiasis lead to a rapid extension of these diseases unless adequate consideration is given to their significance in the planning stages. The introduction of perennial irrigation into Egypt has increased the general incidence of schistosomiasis from 5 to 80 per cent. Dr. D. M. Blair of Southern Rhodesia has advised me that the Umshandige Irrigation Scheme installed in that country at a cost of 3,000,000 pounds in 1939 has practically been abandoned and that the extension of schistosomiasis into the area has contributed materially to the failure of the project.

It is to be hoped that the Seven-Year Plan for the rehabilitation of Iran being engineered by Overseas Consultants, Inc. will give due weight to the malaria problem in the expenditure of $60,000,000 for new irrigation projects in that country. The final report of the United Nations Economic Survey Mission for the Middle East lists a considerable number of irrigation projects which have been projected for flood control purposes and for increased agricultural production. Some of these developments lie in highly endemic areas of schistosomiasis and malaria; unless suitable provisions are made for the control of these diseases (and the reports do not indicate that they have been taken into consideration), economic benefits may be more than offset by the extension of these maladies. In Iraq, the Habbaniyah and the Wadi Tharthar Schemes in the Euphrates and Tigris River valleys, respec-

tively, are located in heavily endemic areas of schistosomiasis. In Syria, the Khabur River Irrigation Scheme is also of importance with regard to the extensions of this disease because of the very high endemic area around Koubour El Bid which is situated in the head waters of this river. The Upper Euphrates River project also offers potentialities for the spread of schistosomiasis, since there is another highly endemic area in Tel-Abiad on the upper reaches of this river.

There seems little likelihood that the steadily increasing world population can be provided with adequate food supplies unless cultivatable lands available in the tropics are eventually exploited. This exploitation will have to go hand in hand with the establishment of sanitation and disease control, not only for the benefit of white settlers but the native populations as well. We shall need to apply all the knowledge presently available and shall also be acutely in need of new knowledge in order to make the tropics a fit place in which to live and produce. Any future research in medical parasitology should serve as a direct contribution to world economy in helping to open up new areas which may be urgently needed in the not far distant future to care for an increasing demand for food supplies.

LOOKING HOMEWARD

If we lower our sights a bit and examine the domestic scene, it is obvious that there are many problems in medical parasitology still awaiting solution on the home front. It is true that malaria is passing and is subject to eradication within the near future. With certain other parasitic diseases we have not done so well.

With the highest incidence of trichina infection of any country in the world, we have made no substantial effort to control the disease. There has been no reduction in the number of clinical cases since data were first recorded by the Public Health Service. Of 11,640 necropsy examinations for the parasite in the United States, 15.4 per cent have been positive. Most effective methods were not employed in many of the surveys; when the search has been more thorough, one in every 6 persons examined at necropsy has been found infected. Incidence of infection in all countries where surveys have been conducted is lower than in the United States, as

TABLE 2.—*Incidence of trichinae in man as revealed by autopsy studies in various foreign countries*

Country	Author	Year	No. examinations	Per cent positive
Australia	Bearup	1937	119	2.3
Canada	Kuitunen-Ekbaum	1941	420	1.7
Chile	Martinic	1944	296	12.5
Czecho-Slovakia	Hökl, Cervinka and Klauz	1940	459	0
England	Van Someren	1937	200	1.0
England	Young	1947	472	10.8
Germany	Wigand	1941	100	0
Germany	Busse	1909	379	6.9
Guatemala	Penagos	1944	200	0
Holland	van der Meer, de Graaf and Brug	1941	1,001	0.2
Portugal	Silva Leitao and Borges Ferreira	1945	13	0
Mexico	Perrin	1939	200	12.5
Mexico	Mazzotti	1943	1,000	7.8

indicated by the data in Table 2. Infection in swine in other countries, as shown in Table 3, is also much lower than the over-all rate of 1.5 per cent for this country, with the possible exception of China and Canada, where only a limited number of swine have been examined. Our record with this disease is nothing to be proud of and we have temporized with it too long.

TABLE 3.—*Incidence of trichinae in swine in various foreign countries*

Country	Author	Year	No. swine examined	Per cent positive
Argentina	Young	1947	589	0
Bulgaria	Matoff	1939	441,392	0.36
Canada	Cameron	1939	2,000	0.75
Canada	Moynihan and Musfeldt	1949	1,067	4.5
China	Riley and Chen	1932	313	0
China	Koo	1941	320	1.5
Denmark	Hjortlund	1935	6,000,000	0.0007
England	Young	1947	4,626	0
Germany	Goldmann	1935	22,000,000	0.00016
Germany	Mayer	1939	100,000	0.001
Guatemala	Padilla	1943	1,190	0
India	Maplestone and Bhaduri	1942	100	0
Poland	Ginsberg	1941	3,604,737	0.05
South Africa	Mönnig	1944	1,352	0
Upper Silesia	Ginsberg	1941	380,088	0.009
Venezuela	Vogelsang	1946	2,000	0
Yugo-Slavia	Debelić	1937	30,000	0.004

In spite of our much vaunted high levels of sanitation, amoebiasis appears to be on the increase. Cases reported to the Public Health Service for the calendar year 1949 represented an increase of more than 62 per cent over the median number reported yearly between 1944 and 1948. For the first time the number of amoebiasis cases exceeded the number of malaria cases. The total of reported amoebiasis cases was greater than the number reported for each of the following diseases: Brucellosis, conjunctivitis, infectious encephalitis, malaria, meningococcal meningitis, rheumatic fever, Rocky Mountain spotted fever, smallpox, tetanus, trachoma, trichinosis, tularemia, typhoid fever, paratyphoid fever, and endemic typhus. The morbidity rate for amoebiasis in Navy personnel in 1948 was three times as high as the peak figure for the war years. If we are to make any progress in the control of this and other intestinal protozoal infections, we must know something more about the epidemiology and manner of spread. A number of staff members in our laboratory have contracted amoebiasis in recent years; in no single instance has it been possible even to surmise where or how infection was acquired. Two of the four members of our Bethesda staff attending the meeting of this Society in New Orleans in 1948 contracted *Giardia lamblia* infections with very annoying clinical symptoms. These two individuals stayed in different hotels and the only time during the meeting when they ate at the same place was at the annual luncheon of this Society.

After 50 years of hookworm control in the Southern States, 15,810 cases of the disease were reported to the Public Health Service for the calendar year 1949. At that, no cases were listed from North Carolina, Alabama or Texas. In spite of improved economic conditions throughout the South, we are all aware that hookworm disease still exists in certain areas, although the incidence of infection has declined materially over the years.

Oxyuriasis can hardly be considered a major health problem, but it is a very annoying condition in children and sometimes in adults. It is difficult to measure the impact of this infection on the health and well being of the individual but it is undoubtedly responsible for certain deviations from the normal. Regardless of the high incidence of the parasite throughout most of the United States, we still lack adequate methods for preventing the transmission of infection.

At various times during World War II, we faced a number of crises because of the exposure of large numbers of troops to various exotic diseases. During the early part of the war, filariasis loomed as a problem in certain of the South Pacific Is-

lands; in New Guinea the attack rate from malaria was so high at one time as to constitute a deterrent to military operations; in New Guinea, as well as in Assam and in the Philippines, scrub typhus was an ever present threat; the invasion of Leyte brought even another problem, schistosomiasis; in Italy epidemic louse-borne typhus in the civilian population was the cause of military concern; and in the Near East sandfly fever was responsible for considerable morbidity in our troops. Each of these threats in turn brought about intensive research on methods of prevention and control, research which proved highly productive. Nevertheless, there was a total of 759,602 cases of parasitic and arthropod-borne diseases in our military forces. In the Navy and Marine Corps alone between 1942 and 1945, these diseases were responsible for 4,709,870 sick days (14). These conditions were costly in terms of military operations, in man days lost, and in medical care. The Veterans Administration has ruled that for compensation purposes veterans may have presumed to have contracted certain tropical diseases during their service if such veterans are found to have these diseases within a year from their discharge, or within the accepted incubation period of the disease. Hence, certain of these diseases are still costing the taxpayer money and will continue to do so for some time to come. I believe it to be an undeniable fact that had our research been pursued more intensively over a period of years prior to the war, we would have been in a much better position to carry on campaigns in tropical areas and could have protected our troops to far better advantage.

We are better prepared now to combat parasitic and other tropical diseases than we were in 1941. However, not all problems in this field have been solved. If we are forced into another widespread conflict, there will be an immediate demand for answers to some of these problems. It would be much better if some of that research could be done before the need arises. Medical parasitologists made outstanding contributions to military preventive medicine during World War II. Because of the unsettled condition of the world today, our interest in this field should not lag in this era of a troublesome peace.

PROBLEMS AWAITING SOLUTION

In spite of accomplishments in the field of medical parasitology over the life span of this Society, there are many problems which remain unsolved.

In chemotherapy, better treatments are needed for amoebiasis, schistosomiasis, onchocerciasis, filariasis, African sleeping sickness, hookworm disease, oxyuriasis, and *Trichuris, Strongyloides* and tapeworm infections. There are available today no specific remedies against trichinosis, toxoplasmosis, Chagas' disease, and paragonimiasis. We are badly in need of a true causal prophylactic and a curative drug for *Plasmodium vivax* infections. Few data are available concerning methods by which any given drug acts on any given parasite. We need to know more about host-parasite-drug relationships in order to develop more effective means of chemotherapy. Knowledge concerning the fate of parasiticides in the animal body would reveal evidence of value in the screening of new compounds.

The life cycle of the human malarial parasites requires more elucidation to rationalize present conflicting views and varying experimental results. The recent findings of Chardome and Peel (15) which cast doubt on Sharp's description of the life cycle of *Acanthocheilonema perstans* and its transmission by *Culicoides* should

serve as a warning that we should not accept previous concepts with complacency and that the critical attitude in science is still to be respected.

The epidemiology of a number of important parasitic diseases awaits clarification. Amoebiasis has been mentioned. Kala-azar is another case in point. In spite of the vast amount of research already conducted, a number of factors surrounding the transmission of the disease are still obscure. Practically nothing is known concerning the mode of transmission of toxoplasmosis, the number of cases of which are increasing as time goes on. There are many undetermined phases in the epidemiology of African sleeping sickness. To mention a few unsolved problems, there is the question of a reservoir host for *Trypanosoma rhodesiense;* the relationship of the density of fly population to the spread of the disease; the effect of temperature and humidity on infection and its speed of development in the fly; the character of the vegetation in relation to tsetse breeding; the relative importance of mechanical transmission; the diurnal and nocturnal feeding habits of various species of *Glossina;* the physiology of digestion in the fly as related to the development of cyclical trypanosomes; reliable criteria for the specific identification of trypanosomes; trypanosome genetics; and more reliable diagnostic techniques. The control of sleeping sickness and the development of the resources of Central Africa depend greatly on answers to these and other questions.

Control measures are lacking for many important parasitic diseases. One could count on the fingers of one hand the localized areas on the globe where any substantial progress has been made in the control of schistosomiasis. Next to malaria, this ranks as the most important tropical disease in the world today. Certain endemic areas of onchocerciasis would be amenable to successful attack provided suitable measures were available. The endemic foci in the Western Hemisphere are not extensive. While DDT has been employed successfully for the destruction of *Simulium* larvae in a number of places, the same methods are not applicable in the difficult terrain in Mexico and Guatemala. *Simulium ochraceum,* which is believed to be the chief vector, breeds almost exclusively in small rivulets high in the mountains and difficult of access. Treatment of such small breeding places by air would be almost wholly ineffective because of the heavy umbrella of vegetation. Streams in the endemic areas have a multitude of tributaries. In one small area in Guatemala, in which a pilot control study is now being conducted, the relatively small stream involved has a total of 355 tributaries, all of which will have to be treated. Even to map such a water course requires a tremendous expenditure of time and energy.

The sleeping sickness problem has already been mentioned. Some measure of control has been achieved in a number of localized areas by various methods, including chemotherapy, resettlement, the intensive application of insecticides, bushing, vegetation control, the hand collection of *Glossina* pupae, and the trapping of adult flies. Some of these methods are extremely costly and others are limited in their usefulness. More research undoubtedly needs to be done before more effective and more universally applicable control measures can be formulated.

Hydatid disease in an important public health problem in many parts of the globe. During recent years, the disease has been controlled to a large extent in Iceland and some progress has been made in New Zealand and Australia. Control procedures have included the suitable disposition of animal viscera and the treatment of dogs

for the removal of the adult tapeworm. A more effective treatment would add to the efficiency of this method of control.

Trichinosis was mentioned as one of our domestic health problems. There are varying opinions as to the feasibility and practicability of various measures which could be applied for the control of the disease. Methods for the destruction of trichinae in pork scraps in garbage as well as in market pork need further investigation. This phase of the problem has received relatively little attention.

The solution of many of these problems lies in a type of fundamental research intimately linked with the collateral physical sciences. We have made a fine beginning in our investigations into the physiology of parasites but nothing more than a beginning. We talk rather eruditely about host-parasite relationships but only the most obvious facts are available to us. We measure such relationships by the crudest of yardsticks. While we know something about the metabolism of the host, our knowledge of the metabolic activities of the parasite is in most cases exceedingly scanty. Lacking adequate knowledge, our insight into the behavior of the parasite within the host and the reactions of the host to the parasite is pretty obscure. In discussing this point, one of our members (16) has recently stated the problem very succinctly:

> "It follows that it is imperative to develop techniques for cultivating intestinal helminths *in vitro*, and to make further study of intestinal physiology. It seems apparent that the cultivation of parasitic helminths outside the host presents one of the *most difficult and most challenging problems facing parasitologists today.*"

When we attempt to evaluate the effect of the parasite on the host, we are guided by obvious signs of symptomatology and pathology. Sometimes we can account for the mechanisms responsible for these phenomena; at other times, such mechanisms are incomprehensible to us. We see the statement many times that toxins elaborated by certain parasites are responsible for damage to the host. What are these so-called toxins? So far as I am aware, a true exotoxin has been described only for *Sarcocystis*. Are these toxins metabolic end-products excreted by the parasite? Presumably, in some cases they are but we certainly know little or nothing about them. For instance, if we could account for the manner whereby *Trypanosoma rhodesiense* brings about the death of the patient, we might have a clearer insight into more rational methods of therapy.

Much has been learned during the past 25 years of the immunology of parasitic diseases yet much remains to be elucidated. The chemical approach has not been pursued intensively and might be the means of adding new facts to our knowledge. More effective and specific immunological tests would be of considerable aid in the diagnosis of certain parasitic diseases.

The phenomenon of drug fastness has been mentioned. Heretofore, the chief concern has been in connection with the treatment of *Trypanosoma gambiense* infections and the resistance which some strains develop to continued treatment with certain arsenicals and antimonials. More recently the marked acquired resistance of West African (17) and Malayan (18) strains of *Plasmodium falciparum* to chlorguanide (paludrine or proguanil), as well as the resistance of *P. vivax* to the same drug (19), has added another problem. The natural resistance of certain strains of houseflies and the acquired resistance of other strains to DDT is a well recognized phenomenon. Resistance to this insecticide is apparently a genetically stable char-

acter but little is known concerning the physiological mechanisms which govern it.

I have mentioned only a few of the fundamental problems which will require for their solution the application of broader knowledge, more diverse talents, and newer and more refined techniques than have been commonly employed by parasitologists in the past. Problems such as these should provide a particular challenge to the younger members of this Society whose research career lies mainly before them. I hope that future parasitologists will avail themselves of more training in complementary disciplines such as biochemistry, physics, physiology, pathology, and pharmacology so that they may be better prepared to tackle the more fundamental and more difficult problems which still await solution. If necessary, I should not feel too badly about sacrificing some training in biology for the sake of acquiring more instruction in collateral sciences.

Medical parasitology is still a fertile field. We may expect to see during the next 25 years further worthwhile contributions from our research endeavors, contributions which will aid in the control of disease and in the betterment of mankind in many parts of the world.

"Men, my brothers, men the workers, ever reaping
something new:
That which they have done but earnest of the
things that they shall do!"

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THE TERMINOLOGY AND CLASSIFICATION OF TROMBICULID MITES (ACARINA: TROMBICULIDAE)

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In a conference on the taxonomy of trombiculid mites at the Rocky Mountain Laboratory, Hamilton, Montana, July 1948, the authors decided that a definition of terms used in descriptions of species is needed and that it would be desirable to indicate those diagnostic characters now considered useful, as well as others of potential value, to an adequate description. Furthermore, the question of acceptable generic names was considered. It was decided that the results of the conference should be published as a joint paper on the terminology and classification of trombiculid mites under the following three sections: 1. Suggested characters to be used in the descriptions of larvae and a glossary of terms. 2. Suggested characters to be used in the descriptions of adults and a glossary of terms. 3. Keys to the genera and subgenera based on larval characters and a key to the genera based on adult characters.

DESCRIPTIVE CHARACTERS OF LARVAE AND GLOSSARY

Larval Descriptions: At present many new species are being described wtih little uniformity; therefore it is difficult to fit the various new forms into the general classification and essential key characters are often lacking. There are still some investigators throughout the world who are basing their descriptions on a series of cotypes (sometimes on the same slide) rather than establishing a holotype and paratypes. For the protection of the describer himself as well as to follow more accepted taxonomic practice for future reference, we feel it cannot be too strongly urged that holotypes be designated along with their depository. Ewing (1949) has attempted to supply the need for uniform terminology by presenting a glossary of terms used in the description of trombiculid larvae. His glossary was not intended to be complete and in places it represents his views rather than general practice. The accompanying scheme will supply sufficient information to place the larva in the system of classification as it is now understood although advances in our knowledge may make this scheme too superficial. A description should ideally include the following characters:

Body. Length, width, shape, color, striae, eyes, ocular plates, spiracles and tracheae, position of anal opening. Degree of engorgement must be considered in the application of most of these characters.

Gnathosoma. Chelicera: basal segment, punctae; pseudochela; distal segment,

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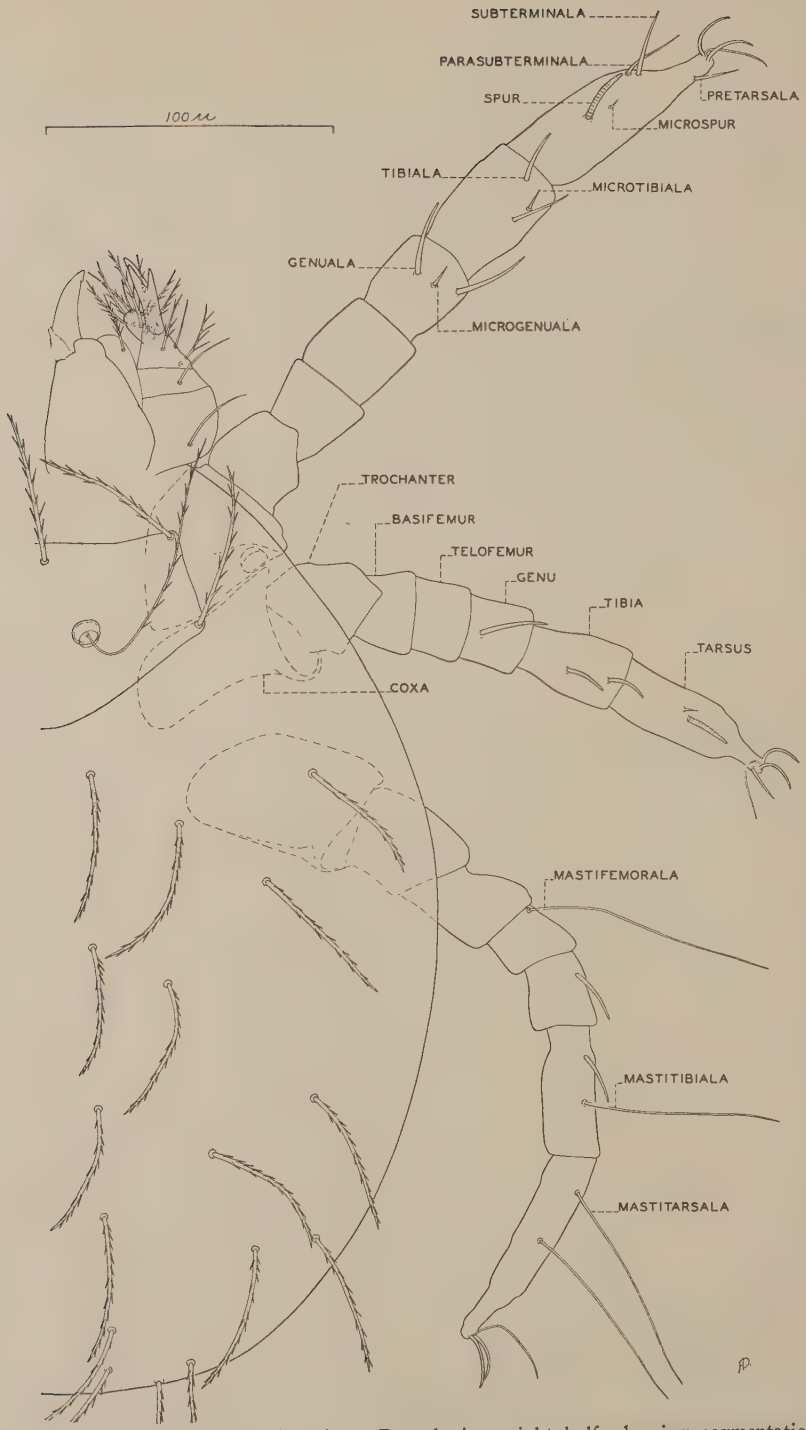

FIG. 1. *Trombicula microti* Ewing. Dorsal view, right half, showing segmentation and specialized setae of the legs. (After Brennan and Wharton 1950).

teeth, tricuspid cap, unusual modifications. Palp: coxa, seta; trochanter; femur, seta; genu, seta; tibia, dorsal seta, lateral seta, ventral seta; palpal claw, axial

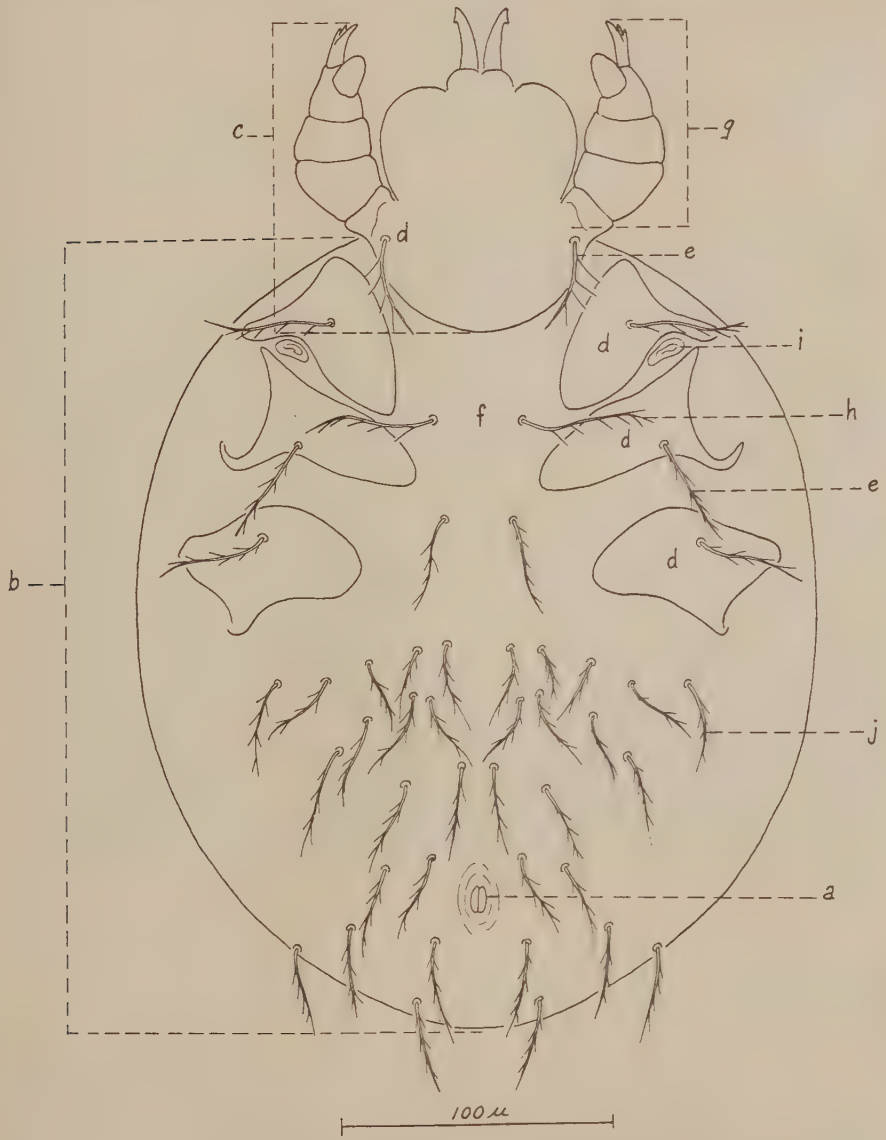

FIG. 2. *Trombicula microti* Ewing. Ventral view. a, anus, b, body, c, gnathosoma, d, coxa, e, coxal seta, f, sternum, g, palp, h, sternal seta, i, urstigma, j, ventral seta.

prong, accessory prongs; tarsus, spur, subterminala, dorsal seta, apical setae, ventral setae; inner lobe of palpal base. Galea, galeal seta.

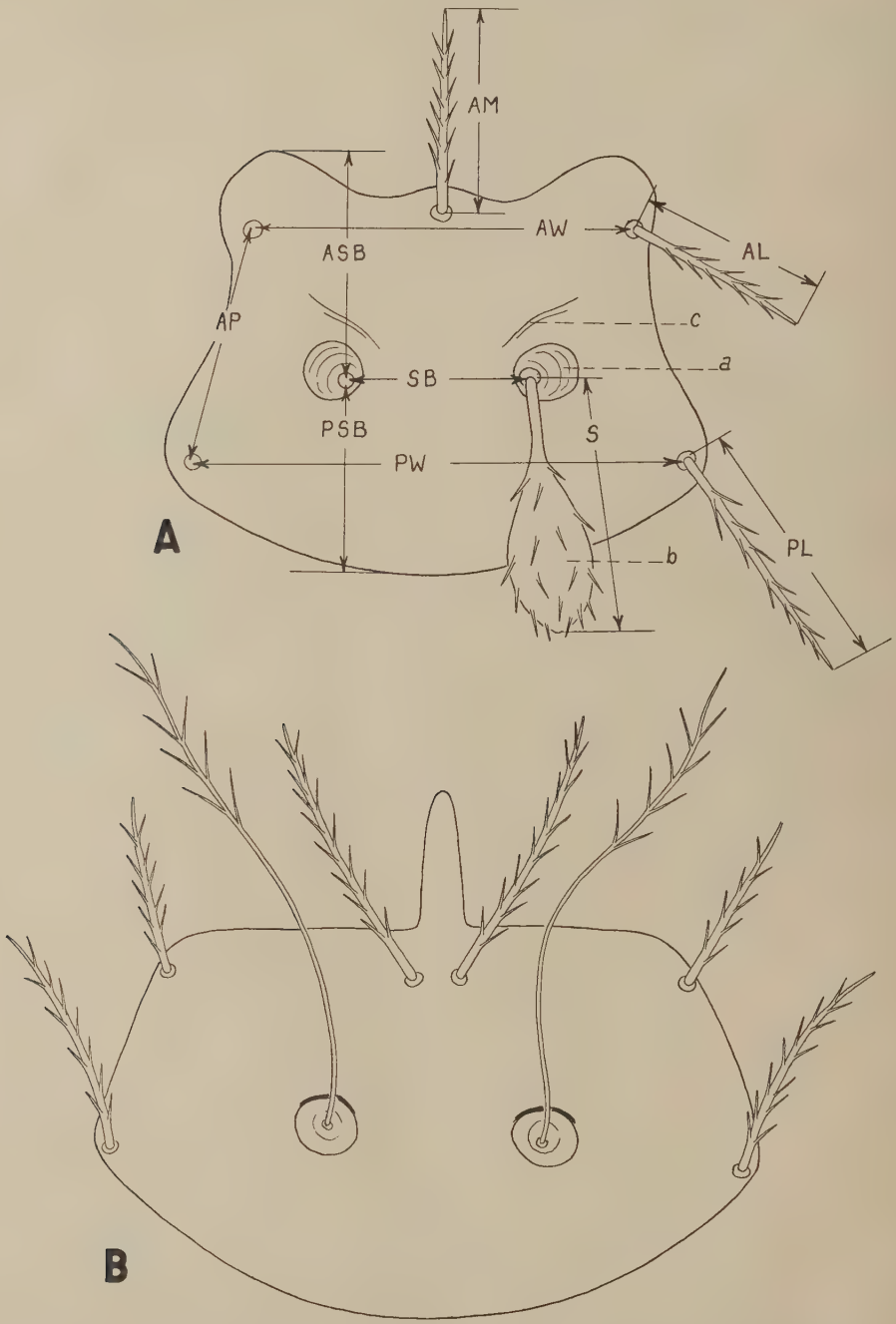

FIG. 3. A. Scutum of *Euschöngastia indica* (Hirst) to show how the standard measurements are taken. a, sensillary base, b, sensilla, c, ridge. (After Wharton 1946).

B. Scutum of *Acomatacarus arizonensis* Ewing to show the submedian setae and the anteromedian projection of the scutum.

Legs. Leg I: coxa, setae; trochanter, setae; femur (if divided, basifemur and telofemur), setae; genu, setae, genulae, microgenualae; tibia, setae, tibialae, microtibialae; tarsus, setae, spur, microspur, subterminala, parasubterminala; pretarsus, setae, pretarsala, claws, empodium. Urstigma, diameter, shape, position. Leg II: coxa, setae; trochanter, seta; femur (if divided, basifemur and telofemur), setae; genu, setae, genualae, microgenualae, tibia, setae, tibialae, microtibialae; tarsus, setae, spur, microspur; pretarsus, setae, pretarsala, claws, empodium. Leg III: coxa, setae; trochanter, setae; femur (if divided, basifemur and telofemur), setae, mastifemorala; genu, setae, genualae, microgenualae; tibia, setae, tibialae, mastitibialae; tarsus, setae, spur, mastitarsalae; pretarsus, setae, claws, empodium.

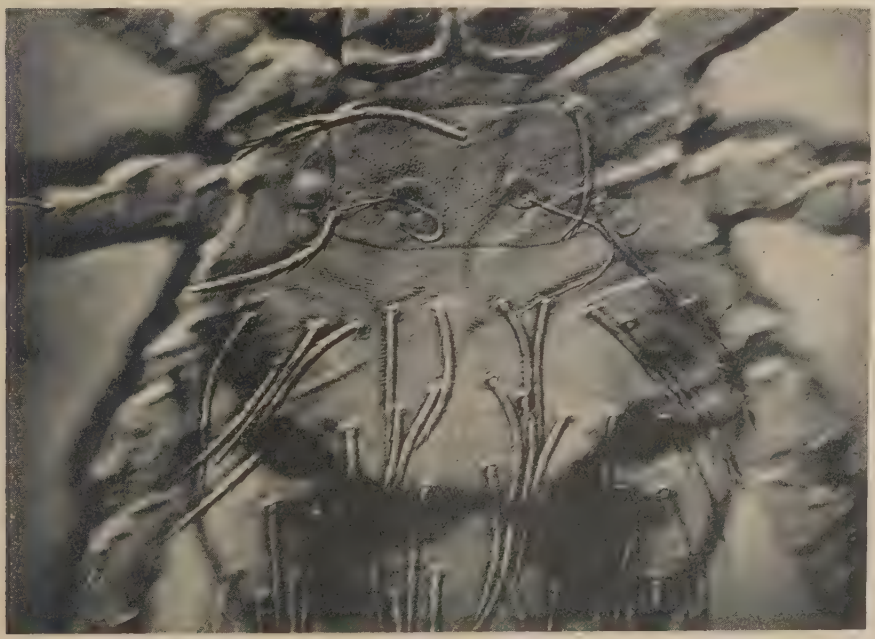

FIG. 4. Photograph of *Trombicula akamushi* (Brumpt) to show the ornamentation on the scutum, slits, eyes on ocular plates lateral to the scutum and striae over the body. (Photo by Kramis, Rocky Mountain Laboratory.)

Scutum. Shape; ornamentation, punctae, striae, ridges, slits; sensillary bases, position, distance of separation; sensillae, shape, armature; setae, shape; anterior median projection. Measurements: AW PW SB ASB PSB AP AM AL PL S (see glossary below for explanations).

Setae (exclusive of scutum and appendages). Dorsum: humeral setae, length, shape, number; dorsal setae, length (of anterior and posterior if different), shape, number; dorsal setal formula. Venter: sternal setae, length, shape, number; ventral setae, length, shape, number; ventral setal formula.

Illustrations. Dorsal and ventral diagrams of body and gnathosoma should be given. If possible, a photograph of the scutum, or at least a drawing should be included, as well as drawings of any other characters of diagnostic value.

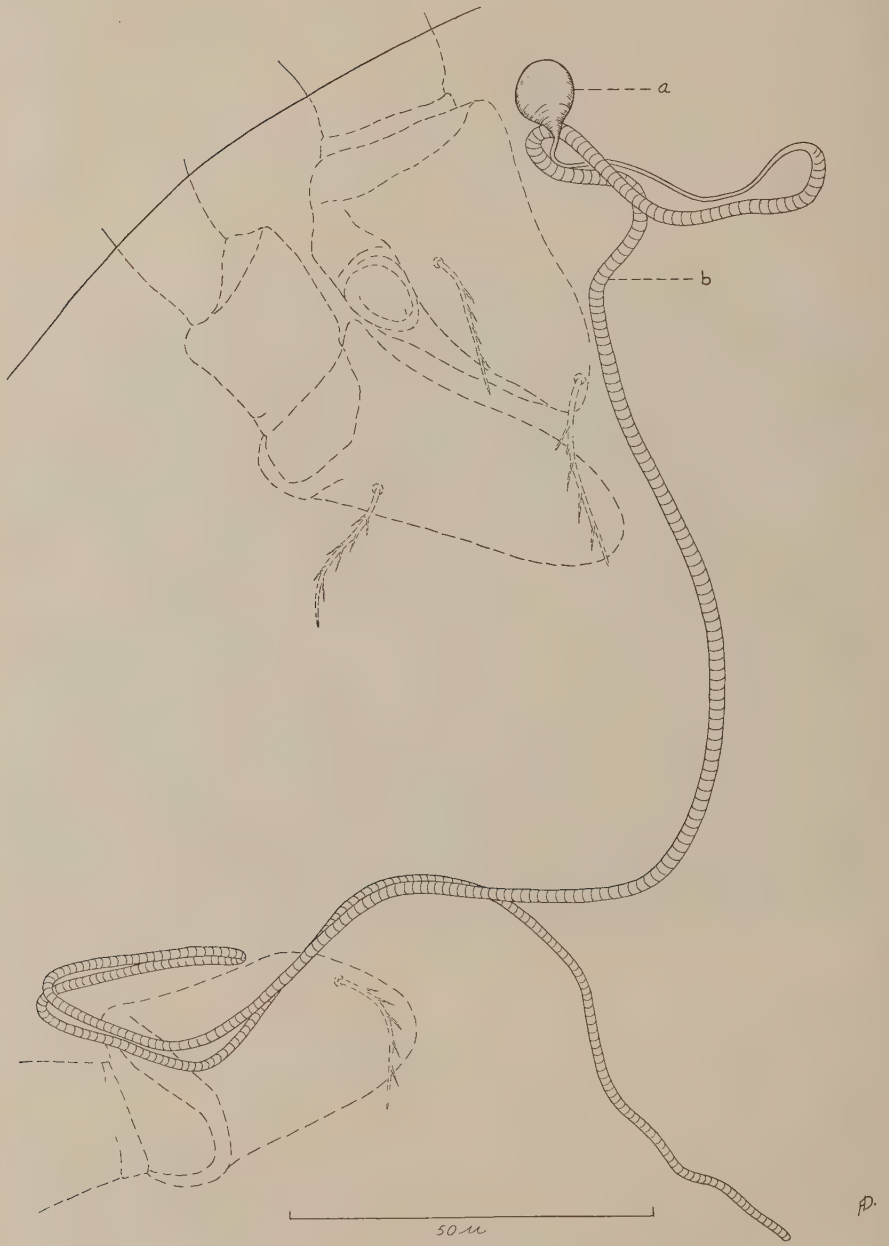

FIG. 5. Spiracle and anterior portion of trachea of *Acamatacarus arizonensis* Ewing. a, stigma, b, trachea. (After Brennan 1949.)

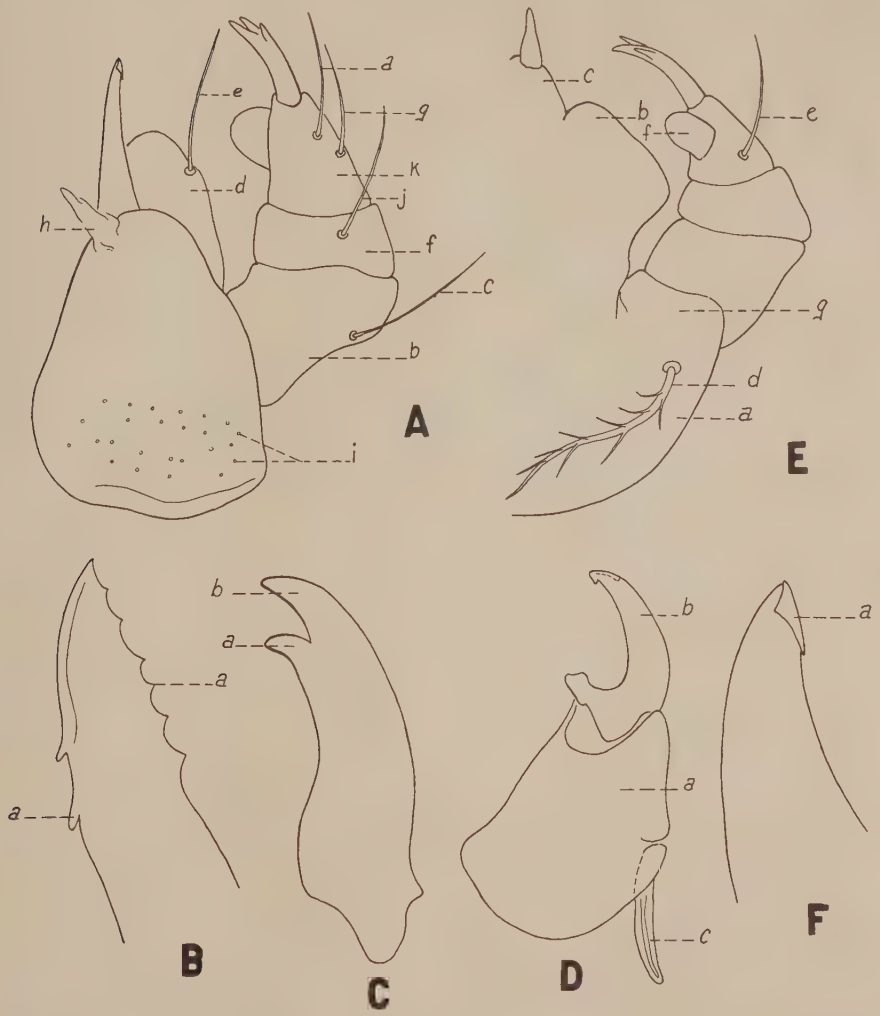

FIG. 6. The gnathosomal structures of trombiculids. A. Dorsal view of gnathosoma, right half. a, dorsal tibial seta, b, palpal femur, c, femoral seta, d, galea, e, galeal seta, f, genu, g, lateral tibial seta, h, pseudochela, i, punctae, j, genual seta, k, tibia. B. Blade of the chelicera of *Acomatacarus*. a, teeth. C. Palpal claw of *Trombicula splendens* Ewing. a, accessory prong, b, axial prong. D. Chelicera. a, basal segment, b, blade or distal segment, c, apodeme. E. Ventral view of gnathosoma, left half. a, capitular sternum, b, galea, c, inner lobes of palpal base, d, coxal seta, e, ventral tibial seta, f, palpal tarsus. F. Blade of the chelicera. a, tricuspid cap.

GLOSSARY (measurements in microns)

Accessory prongs. Projections from the palpal claw that diverge from the main axis of the claw. Figure 6Ca.

AL. An abbreviation in describing the scutum for the length of the anterolateral scutal seta. Figure 3A.

AM. An abbreviation in describing the scutum for the length of the anteromedian scutal seta. Figure 3A.

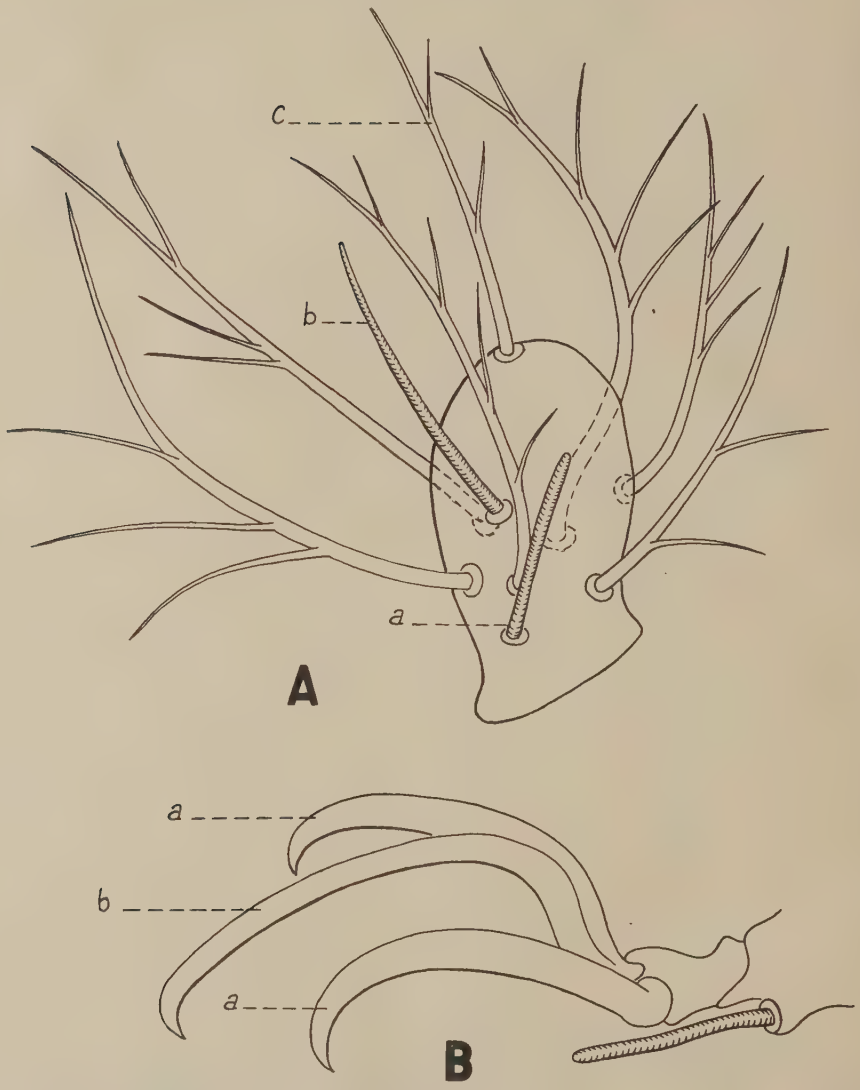

FIG. 7. A. Palpal tarsus of *Trombicula splendens* Ewing. a, spur, b, subterminala, c, apical seta.

B. Pretarsus of *Trombicula splendens* Ewing. a, claw, b, empodium.

Anal opening. The external opening from the hind gut. It is located along the median line in the posterior third of the body on the ventral surface. Figure 2a.

Anteromedian projection. An extension of the scutum from the anterior margin at the mid-line that is usually tongue-shaped. Figure 3B.

AP. An abbreviation in describing the scutum for the distance between the bases of the anterolateral and posterolateral scutal setae. Figure 3A.

Apical seta. The seta on the distal end of the palpal tarsus. Usually only one is present. Figure 7Ac.

ASB. An abbreviation in describing the scutum for the distance from the anterior margin of the scutum to the level of the sensillary bases. Figure 3A.

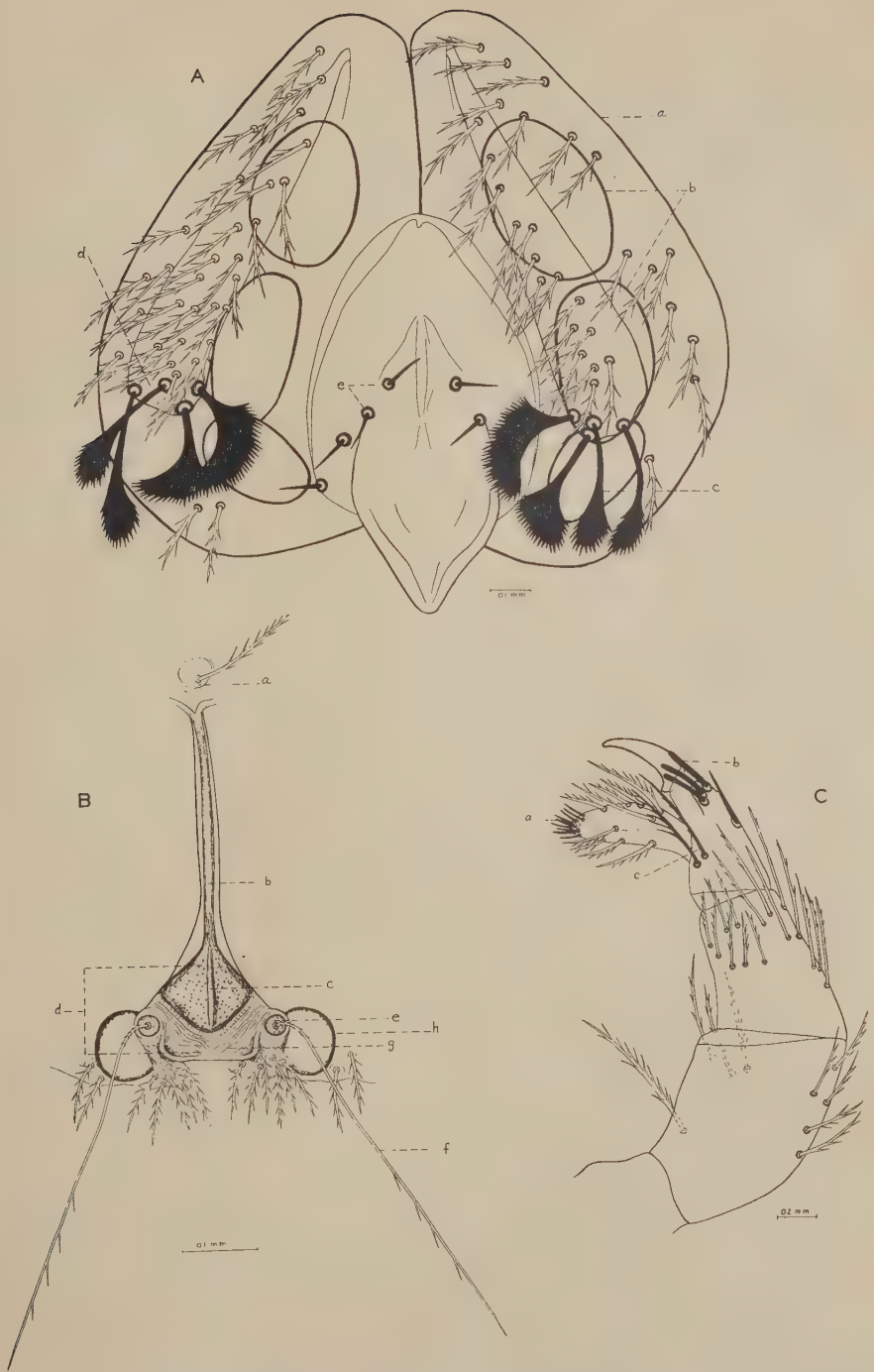

FIG. 8. A. Genital area of male of *Trombicula splendens* Ewing. a, genital plate, b, genital suckers, c, genital seta, d, genital papilla, e, penile setae. (After Jenkins 1949.)

B. Scutum of adult of *T. splendens*. a, tectum, b, crista, c, carina, d, sensillary area, e, sensillary base, f, sensilla, g, saddle, h, eye. (After Jenkins 1949.)

C. Inner view of palp of adult of *T. splendens*. a, apical tarsal setae, b, ctenidia, c, inner tibial setae at tarsal articulation. (After Jenkins 1949.)

AW. An abbreviation in describing the scutum for the distance between the bases of the anterolateral setae. Figure 3A.

Axial prong. The terminal element of the palpal claw that is in line with the long axis of the claw. Figure 6Cb.

Barb. A hair-like projection from a seta or sensilla.

Basal segment of the chelicera. The free segment of the chelicera that is attached to the idiosoma. It bears the distal segment or blade of the chelicera. Figure 6Da.

Basifemur. The proximal portion of the femur when the femur is divided. Figure 1.

Body. The region of the mite posterior to the gnathosoma exclusive of the appendages. See idiosoma. Figure 2b.

Capitular sternum. The fused elements that form the proximal ventral wall of the gnathosoma. Hypostome of some authors. Figure 6Ea.

Capitulum. The anterior, sclerotized region of the mite, consisting of the palpi, chelicerae, and supporting and protective structures of the latter. See gnathosoma. Figure 2c.

Caudal plate. A posterior sclerotized region characteristic of the genus *Guntherana.*

Chelicera. The inner paired appendage on the capitulum which consists of three parts: apodeme, basal segment, and piercing blade. Figure 6D.

Cheliceral apodeme. A sclerotized rod that is attached to the ventroposterior angle of the basal segment and to which muscles are attached. Figure 6Dc.

Cheliceral blade. The distal segment of the chelicera. It is the piercing organ and is usually provided with a tricuspid cap and in some groups with series of teeth or other modifications. Figures 6Db, 6B and 6F.

Claws. The paired, lateral elements on the distal end of the pretarsi of the legs. The empodium is also included by some who describe species as "3-clawed." Figure 7Ba.

Color. May vary from white to deep scarlet. No satisfactory method of reporting the color has been regularly used.

Coxa. The proximal segment of an appendage. The palpal coxae are fused with each other medially and with their trochanters laterally. The chelicerae are so modified that the primary segmentation cannot be determined. The coxae of the legs are immovably joined to the ventral surface of the body. Figure 2d.

Coxal setae. There is a single seta on the coxa of the pedipalps and at least one on each coxa of the legs. In the Leeuwenhoekiinae coxa I bears two setae. More than one seta may be present on the other coxae in other groups, especially coxa III. Figure 2e.

Dorsal seta. A seta on the dorsal surface of the body. These setae are usually arranged in transverse rows and the number in each row is fairly constant within many species. Relative lengths of anterior and posterior setae may vary. The dorsal setal formula may depend upon the degree of engorgement of an individual specimen. Figure 1.

Dorsal setal formula (DS). A notation for indicating the arrangement of the dorsal setae. The number of setae in row 1 is followed by the number in 2, 3, etc. to the posterior limit of the body, though posterior rows are sometimes confused. The formula for the specimen shown in figure 1 is: 2–6–6–6–4–4–4.

Dorsal tibial seta. Of the three setae on the palpal tibia (article IV of some), this is located on the medial dorsal surface (palpal seta 5 in Ewing's system of numbering the palpal setae). Figure 6Aa.

Empodium. The thin, usually claw-like, median extension of the pretarsus. Figure 7Bb.

Eyes. Usually paired ocelli in tandem, lateral to the scutum, frequently on an ocular plate. In some species they are greatly reduced or absent. Figure 4.

Femur. The third segment of the appendages. The palpal femur is the first movable segment of the palp (article II of some). The femur of the leg is frequently divided into a basifemur and telofemur. Figures 1 and 6Ab.

Femoral seta. The palpal femur bears a single seta on its dorsal aspect (palpal seta number 1 according to Ewing's notation). Figure 6Ac.

Galea. An anterolateral lamellate projection of the ventral wall of the gnathosoma that curls around the chelicera on each side. It bears a single seta anteriorly. Figures 6Ad and 6Eb.

Galeal seta. The seta on the anterolateral face of the galea. Figure 6Ae.

Genu. The segment of the appendages just distal to the femur. On the palp the genu bears a single seta (article III of some). The genua of the legs bear specialized sensory setae as well as setae of the ordinary type. Figures 1 and 6Af.

Genuala. Specialized nude seta on the genu. Figure 1.

Genual seta. The seta on the dorsal aspect of the palpal genu (palpal seta 2 according to Ewing's notation). Figure 6Aj.

Gnathosoma. The region of the mite consisting of the palpi, chelicerae, and the supporting and protective structures of the latter. Synonymous with capitulum but a more widely used term in describing trombiculid species. Figure 2c.

Humeral setae. Dorsal setae lateral and anterior to the dorsal setae that form continuous rows on the idiosoma. Most species have a single pair, but multiple humeral setae are present in a few species of some genera. Figure 1.

Idiosoma. The portion of the mite posterior to the gnathosoma. See body. Figure 2b.

Inner lobes of palpal base. An anteromedian, ventral, extension of the ventral wall of the gnathosoma. Figure 6Ec.

Intercoxal area. The area on the ventral surface between the coxae. See sternum. Figure 2f.

Interpalpal setae. The setae on the palpal coxae. Figure 6Ed.

Lateral tibial seta. The lateral seta on the palpal tibia (palpal seta number 4 of Ewing). It may be displaced dorsally or ventrally in some species. Figure 6Ag.

Legs. The ambulatory appendages. They consist of the following segments: coxa, trochanter, femur (may be divided into basifemur and telofemur), genu, tibia, tarsus, and pretarsus. The pretarsus usually bears a pair of lateral claws and a median claw-like empodium. Figure 1.

Length. The distance from the posterior end of the body to the anterior tip of the gnathosoma.

Mastifemorala. A flagelliform seta found on the femur. Figure 1.

Mastitarsala. A flagelliform seta found on the tarsus. Figure 1.

Mastitibiala. A flagelliform seta found on the tibia. Figure 1.

Microgenuala. A nude microsensory seta on the genu. Figure 1.

Microspur. A nude microsensory seta found near the spur on tarsi I and II. Figure 1.

Microtibiala. A nude microsensory seta on the tibia. Figure 1.

Ocular plate. A sclerotized region that contains the eyes. Figure 4.

Ornamentation. The markings found on the sclerotized regions of the integument usually consisting of a pattern of punctae or pits especially on the scutum. Figure 4.

Outer lobe of palpal base. The galea. Figure 6Ad.

Palp. The lateral paired appendage of the gnathosoma. It consists of the following movable segments: femur, genu, a terminal tibia with a tibial claw, and a subterminal tarsus. Figure 2g.

Palpal base. The fused palpal coxae and other elements that form the ventral mass of the gnathosoma. Figure 6Ea.

Palpal claw. The claw on the palpal tibia. It has no muscular attachments and is probably a modified seta. Figure 6C.

Parasubterminala. A specialized seta closely associated with the subterminala on tarsus I. It is proximal to and smaller than the subterminala. Figure 1.

Pedipalp. The second appendage and all of its processes. The palp, palpal base, galea, and inner lobes of palpal base are all formed at least in part from the pedipalp. Figure 6E.

PL. An abbreviation, in describing the scutum, for the length of the posterolateral scutal setae. Figure 3A.

Pretarsala. A specialized seta on the pretarsus that is found on legs I and II. Figure 1.

Pretarsus. The terminal portion of the tarsus. It bears the empodium, claws, and pretarsala if present. Figure 7B.

PSB. An abbreviation, in describing the scutum, for the distance from the level of the sensillary bases to the posterior margin of the scutum. Figure 3A.

Pseudochela. A membranous dorsal extension of the basal segment of the chelicera that is present in some species. Figure 6Ah.

Pseudostigmata. A pair of large pits in the scutum that bear sensillae or pseudostigmatic organs. See sensillary bases. Figure 3Aa.

Pseudostigmatic organs. The setae that arise from the pseudostigmata or sensillary bases. See sensillae. Figure 3Ab.

Punctae. Pits that occur on the scutum and the more heavily sclerotized portions of the integument of the appendages. Figures 4 and 6Ai.

PW. An abbreviation, in describing the scutum, for the distance between the bases of the posterolateral scutal setae. Figure 3A.

Ridges. Elevated areas on the scutum that are usually associated with the sensillary bases. Figure 3Ac.

Rostral collar. The collar formed about the chelicerae by the galeae. Figures 6Ad and 6Eb.

S. An abbreviation, in describing the scutum, for the length of the sensillae or pseudostigmatic organs (Sens. of Womersley). Figure 3A.

SB. An abbreviation, in describing the scutum, for the distance between the sensillary bases. Figure 3A.

Scutum. A dorsal sclerotized plate on the body that usually bears five setae and a pair of sensillary bases or pseudostigmata. Figures 3 and 4.

Sensillae. Specialized seta-like structures that arise from the sensillary bases. See pseudostigmatic organs. Figure 3Ab.

Sensillary bases. A pair of pits found on the scutum. See pseudostigmata. Figure 3Aa.

Sensillum. A term used by Ewing for a specialized seta, for example a genuala, spur, subterminala or others.

Setae. The setae or hairs are of several types. Those on the body are usually unmodified, except for the sensillae. On the appendages there are numerous unmodified setae as well as specialized setae.

Seta 1. The seta on the palpal femur, according to Ewing. Figure 6Ac.

Seta 2. The seta on the palpal genu, according to Ewing. Figure 6Aj.

Seta 3. The ventral seta on the palpal tibia, according to Ewing. Figure 6Ee.

Seta 4. The lateral seta on the palpal tibia, according to Ewing. Figure 6Ag.

Seta 5. The dorsal seta on the palpal tibia, according to Ewing. Figure 6Aa.

Setule. A hair-like projection from a seta or sensilla. See barb.

Slits. Slit-like markings occur on the scutum of some species. Figure 4.

Spiracle. The external opening into the tracheae. See stigmata.

Spur. A stout nude striated seta found on the tarsus. It is present on the palp and legs I and II of all species, but is almost always absent from leg III. Figures 1 and 7Aa.

Sternal setae. The setae on the sternum or intercoxal area. Usually there are two pairs present, but in a few species there are many sternal setae and in the Leeuwenhoekiinae the anterior pair of sternal setae are lacking. Figure 2h.

Sternum. The area between the coxae on the ventral surface (intercoxal area of Ewing). Figure 2f.

Stigmata. The external openings into the tracheae. The openings may be between coxa I and the gnathosoma or they may be at the base of the chelicerae. Figure 5a.

Striae. These are the fine lines that make a pattern over the softer portions of the integument of the entire body. They sometimes also encroach on the scutum. Figure 4.

Submedian setae. A pair of anteromedian scutal setae. Figure 3B.

Subterminala. A specialized seta found near the tip of the tarsus on Leg I, and commonly on the palpal tarsus as well. Figures 1 and 7Ab.

Tarsus. The terminal segment of the legs and palps. The palpal tarsus is displaced ventrally however so that it opposes the palpal claw in thumb-like fashion. Figures 1, 6Ef and 7A.

Teeth. Sharp, pointed, irregularities on the cheliceral blade. Figure 6Ba.

Telofemur. The distal portion of the femur when the femur is divided into two sections. Figure 1.

Tibia. The penultimate segment of the appendages. Figures 1 and 6Ak.

Tibiala. A specialized nude seta that is found on the tibia of the legs. Figure 1.

Tracheae. Tubes that are supported by taenidia. Part of the respiratory apparatus. Figure 5b.

Tricuspid cap. The distal portion of the cheliceral blade modified to form three teeth: apical, dorsal, and ventral. Figure 6Fa.

Trochanter. The second segment of the appendages. The palpal trochanters are fused with the palpal coxae in some groups and with the palpal femora in others. Figures 1 and 6Eg.

Urstigma. A large conspicuous pit between coxae I and II. It is of unknown function. Figure 2i.

Ventral Setae. The setae on the ventral surface, including the sternal setae, but not including the coxal setae. The setae posterior to the anus are the same type as the dorsal setae. Figure 2j.

Ventral setal formula (VS). A system of notation that represents the number and distribution of the ventral setae. The number of setae in the first row is followed by the number in the second and so on. The formula for the specimen shown in figure 2 is: 2-2-10-8-4-4-6-2.

Ventral tibial seta. The seta on the ventral aspect of the palpal tibia (palpal seta number 3 of Ewing). Figure 6Ee.

Width. The maximum width of the specimen.

DESCRIPTIVE CHARACTERS OF ADULTS AND GLOSSARY

The study of adult TROMBICULIDAE has progressed much more slowly than that of larvae. Most of the species are known only from the larva and the differentiation

of genera and species is based almost entirely on the characters of this more readily collected stage. Only a few adults are known and in very few species have they been correlated with larvae. Separation of species on the basis of adult characters is a recent advance.

Adults were first described by Berlese (1888) who later (1912) described several on the basis of size, presence of eyes, variation in shape and proportions of the tibia and tarsus I. Nagayo *et al.* (1916) described the adult of Trombicula (*Leptotrombidium*) *akamushi* (Brumpt) and correlated it with the larva, nymph, and other stages. Ewing (1926) described several adults and reared *Trombicula* (*Eutrombicula*) *alfreddugèsi* to the adult stage. Adult characters used for differentiating species were structure and shape of the scutum, the sensillae, and body setae. Womersley and Heaslip (1943) described several adults from the Australian and southwest Pacific area and emphasized measurements of size of body, scutum, sensillae, body setae, and legs. Michener (1946) described adults of several species from Panama and correlated them with larvae. He differentiated species by the number of ctenidial spines on the palpal tibia, type and length of body setae, proportion and shape of tarsus I and tibia I, proportions of scutum and eyes, and length of palpal tibial claw. A key was presented for four *Megatrombicula* occurring from Panama to Peru. Jenkins (1949) reared and correlated *Eutrombicula* adults with larvae and gave a key for four species of adults in the American hemisphere, using the number of apical nude setae on the palpal tarsus, the width between sensillae, length and type of body setae, diameter of eyes, length of sensilla, and relative length of tarsus and tibia I. Adult as well as larval characters were used in retaining or synonymizing certain species.

No uniform procedure for describing adults has been followed in the past, so that comparisons of different genera and species are often impossible unless types are available. The nymphs resemble the adults to a remarkable extent and in the older literature some of the descriptions are not sufficiently detailed to enable the reader to decide whether a nymph or an adult was described. Nymphs differ from the adults in that they are smaller, have fewer setae, have 2 instead of 3 pairs of genital suckers and lack the secondary sexual characters of the adults. Nymphs should be described in the same manner as adults. In order to establish uniformity and to provide complete descriptions of these stages the following structures should be described.

Body. Length, width, shape, color, eyes, anus.

Genitalia. Genital plates and suckers. Male: genital papillae; number, shape and branching of genital setae; penis and penile setae. Female: genital setae, number; genital rings (sacculi), location.

Gnathosoma. Chelicera. Galea. Palp: femur, vestiture; tibia, number and arrangement of ctenidia, number and type of inner setae at tarsal articulation, length and shape of claw; tarsus, number of apical nude setae, relative proportions of different segments.

Legs. Length; number of segments; coxal and precoxal plates and sternum; length, width and shape of tibia I and tarsus I; type and number of tarsal claws.

Scutum. Shape and ornamentation; length, including crista to posterior crests of saddle in sensillary area; tectum and tectal setae; median carina of scutum; shape of sensillary area; shape of saddle bridging the sensillary area between the sensillary

bases, type of posterior lobes on the saddle; distance between sensillary bases; type of sensillae, number of branches and length.

Setae. Type; shape; branching and length of posterior, dorsal and humeral body setae.

Glossary

Carina. A keel-like projection arising from the depressed surface inside the sensillary area and crista. Figure 8Bc.

Crista. The anterior extension of the scutum, sometimes narrowed and rod-like. Figure 8Bb.

Ctenidia. Combs of flat spines or straps located mediodistally on the palpal tibiae. Figure 8Cb.

Genital papillae. A pair of posteromedial projections from the genital plates of the males. Figure 8Ad.

Genital plates. A pair of reniform plates lateral to the genital opening. Figure 8Aa.

Genital rings (sacculi). A pair of small rings located between the genital plates of the female.

Genital setae. Specially modified setae located on the genital papillae of males, or on the genital plates of females. Figure 8Ac.

Genital suckers. Three pairs of oval or egg-shaped structures located on or near the genital plates. Figure 8Ab.

Penile setae. Specially modified spine-like setae located on the penis of the male. Figure 8Ae.

Saddle. A bridge-like fold over the sensillary area between the two lateral sensillary bases. The saddle is usually extended posteriorly into dorsolateral crests or lobes. Figure 8Bg.

Scutum. A sclerotized structure, located anteromedially on the dorsal body surface, which consists anteriorly of a narrowed crista and tectum and posteriorly of an expanded sensillary area. Figure 8B.

Sensillae (pseudostigmatic organs). Specialized seta-like structures arising from the sensillary bases on the scutum. Figure 8Bf.

Sensillary area (pseudostigmatic area). A posterior expansion of the scutum in the region of the sensillary bases. Figure 8Bd.

Sensillary bases (pseudostigmata). A pair of circular depressions on the posterior expansion of the scutum bearing the sensillae. Figure 8Be.

Tectum. A thin plate-like extension of the crista that projects over the gnathosoma and usually bears a single seta. Figure 8Ba.

KEY TO THE SUBFAMILIES AND GENERA OF TROMBICULIDAE BASED ON LARVAL CHARACTERISTICS

1		All legs with six segments, two setae on coxa I (Leeuwenhoekiinae)	4
		First pair of legs with seven segments	2
2	(1)	Legs II and III with six segments (Walchiinae)	9
		Legs II and III with seven segments	3
3	(2)	Paired submedian anterior scutal setae or anteromedian projection of scutum or both present (Apoloniinae)	14
		Anteromedian projection and submedian setae absent (Trombiculinae)	16

Subfamily Leeuwenhoekiinae Womersley, 1944

4	(1)	Chelicerae with teeth restricted to the tricuspid cap	5
		Chelicerae with teeth on the blade	6
5	(4)	With an anteromedian projection on the scutum *Leeuwenhoekia* Oudemans, 1911	
		Type *Heterothrombidium verduni* Oudemans, 1910	
		(See later key to subgenera)	
		No anteromedian projection on the scutum *Chatia* Brennan, 1946	
		Type *Chatia setosa* Brennan, 1946	
6	(4)	With a single anteromedian scutal seta *Odontacarus* Ewing, 1929	
		Type *Trombicula dentata* Ewing, 1925	
		With paired submedian anterior scutal setae	7
7	(6)	No anteromedian projection of the scutum *Whartonia* Ewing, 1944	
		Type *Hannemania nudosetosa* Wharton, 1938	
		Anteromedian projection of the scutum present	8
8	(7)	Distal segment of the chelicerae blade-like, with teeth of various types *Acomatacarus* Ewing, 1942	
		Type *Acomatacarus arizonensis* Ewing, 1942	
		(See later key to subgenera)	

Cheliceral blade expanded distally, greatly modified, with a series of teeth on the expanded portion (parasitic only on Amphibia) *Hannemania* Oudemans, 1911
Type *Heterothrombidium hylodeus* Oudemans, 1910

Subfamily WALCHIINAE Ewing, 1946

9 (2) Anteromedian scutal seta present 10
Anteromedian scutal seta absent 11

10 (9) Five setae present on scutum (*New Genus* Fuller, in press)
Type *Trombicula oudemansi* Walch, 1922
Three scutal setae; posterolateral setae not on scutum (*New Genus* Lipovsky, in ms.)
(Type N. g. n. sp. Lipovsky, in ms.)

11 (9) With four scutal setae *Walchia* Ewing, 1931
Type *Trombidium glabrum* Walch, 1927 (= *Walchia ewingi* Fuller, 1949)
With more than four scutal setae 12

12 (11) With six scutal setae *Schöngastiella* Hirst, 1915
Type *Schöngastiella bengalensis* Hirst, 1915
With more than six scutal setae 13

13 (12) Scutal setae all marginal *Gahrliepia* Oudemans, 1912
Type *Typhlothrombium nanus* Oudemans, 1910
Scutal setae in part not marginal *Gateria* Ewing, 1938
Type *Gahrliepia fletcheri* Gater, 1932

Subfamily APOLONIINAE Wharton, 1947

14 (3) With paired submedian anterior scutal setae 15
With a single median anterior scutal seta *Womersia* Wharton, 1947
Type *Womersia strandtmani* Wharton, 1947

15 (14) With four scutal setae and filiform sensillae *Apolonia* Torres and Braga, 1938
Type *Apolonia tigipioensis* Torres and Braga, 1938
With six scutal setae and capitate sensillae *Sauracarella* Lawrence, 1949*
Type *Sauracarella whartoni* Lawrence, 1949

Subfamily TROMBICULINAE Ewing, 1929

16 (3) Empodium swollen and expanded distally 16A
Empodium claw-like 17

16A (16) Empodium with a spatulate tip *Riedlinia* Oudemans, 1914
Type *Riedlinia coeca* Oudemans, 1914
Empodium with a pulvilliform tip *Mackiena* Traub and Evans, 1950
Type *Mackiena empodiformia* Traub and Evans, 1950

17 (16) Cheliceral blade with several terminal or subterminal processes 18
Cheliceral blade sword-like, not with terminal or subterminal processes (tricuspid cap or teeth may be present) 19

18 (17) With eyes *Oenoschöngastia* Womersley and Kohls, 1947
Type *Oenoschöngastia cana* Womersley and Kohls, 1947
Without eyes *Myotrombicula* Womersley and Heaslip, 1943
Type *Myotrobicula vespertilionis* Womersley and Heaslip, 1943

19 (17) With nine scutal setae *Heaslipia* Ewing, 1944
Type *Trombiculoides gateri* Womersley and Heaslip, 1943
With less than nine scutal setae 20

20 (19) Sensillae expanded distally 21
Sensillae flagelliform 27

21 (20) Caudal plate present *Guntherana* Womersley and Heaslip, 1943
Type *Neoschöngastia kallipygos* Gunther, 1939
Caudal plate absent 22

22 (21) Scutum partially submerged beneath cuticular striae (usually parasitic on birds) *Neoschöngastia* Ewing, 1929
Type *Schöngastia americana* Hirst, 1921
Scutum on surface of integument 23

23 (22) Coxa II with more than one seta *Doloisia* Oudemans, 1910
Type *Doloisia synoti* Oudemans, 1910
Coxa II with a single seta 24

24 (23) Cheliceral blade with a single dorsal tooth 25
Cheliceral blade with several dorsal teeth 26

25 (24) Posterolateral setae on scutum *Euschöngastia* Ewing, 1938
Type *Euschöngastia americana* Ewing, 1938

* Placement of this genus in the Apoloniinae is provisional.

Synonym of *Schöngastia sciuricola* Ewing, 1925
Posterolateral setae not on scutum — *Ascoschöngastia* Ewing, 1946
Type *Neoschöngastia malayensis* Gater, 1932

26 (24) Cheliceral blade with three to six large, prominent, recurved, dorsal hooks, that increase in size proximally — *Endotrombicula* Ewing, 1931
Type *Endotrombicula penetrans* Ewing, 1931
(See later key to subgenera)
Cheliceral blade with a series of dorsal teeth that diminish in size proximally
Schöngastia Oudemans, 1910
Type *Thrombidium vandersandei* Oudemans, 1905

27 (20) With seven scutal setae — *Novotrombicula* Womersley and Kohls, 1947
Type *Novotrombicula owiensis* Womersley and Kohls, 1947
With less than seven scutal setae — 28

28 (27) With five scutal setae — *Trombicula* Berlese, 1905
Type *Trombicula minor* Berlese, 1905
With three scutal setae — 29

29 (28) Chelicerae with a series of minute dorso-apical teeth, palpal claw with four prongs
Tecomatlana Hoffmann, 1947
Type *Tecomatlana sandovali* Hoffmann, 1947
Chelicerae without a series of minute dorso-apical teeth, palpal claw with less than four prongs — 30

30 (29) Palpal claw with two prongs — *Sauriscus* Lawrence, 1949*
Type *Sauriscus ewingi* Lawrence, 1949
Palpal claw with three prongs — *Trisetica* Traub and Evans, 1950*
Type *Trisetica melvini* Traub and Evans, 1950

The genus *Crotiscus* Ewing 1944 is omitted from the above key as one of us (G.W.W.) has examined the cotypes of *Trombicula desdentata* Boshell and Kerr, the genotype, and found them to be congeneric with the genus *Trombicula* and will key to *Trombicula* sensu lato as characterized below in couplet 5.

Keys to the Subgenera

Trombicula.

Trombicula minor Berlese, 1905 is known only from the adult.† Berlese's original description and Willmann's 1941 redescription of the type specimen of *T. minor* indicate that *T. minor* is congeneric with the adults of larvae that will key to the genus *Trombicula* in the above key. Many genera have been proposed for larvae that are placed in *Trombicula.* As yet it is impossible to be sure of the exact type of larva that will be characteristic of *T. minor* (Philip and Traub 1950, and others). Therefore, pending clarification and in order to maintain stability in the use of *Trombicula* as a generic name the subdivisions of this originally large genus are here considered as subgenera. The only other alternative would be to consider *Trombicula* as monotypic until such time as additional information is obtained. Such a course is entirely unnecessary since, despite the plethora of generic names proposed, *Trombicula* as understood here is no broader in its content than are such genera as *Neoschöngastia* and *Euschöngastia.* In handling nomenclatorial problems of this kind, stability should be maintained if it is at all possible to do so and remain consistent with the biological facts in the case.

1 Anterolateral setae short, peg-like — *Fonsecia* Radford, 1946
Type *Trombicula ewingi* Fonseca, 1932

* Possibly synonyms of *Tecomatlana* Hoffmann 1947, but not so considered here because of insufficient evidence.

† According to recent information received from Dr. Willmann through Dr. C. E. M. Gunther by personal communication, the types of *T. minor* were destroyed as a result of bombing in World War II.

Anterolateral setae elongate, and barbed or feathered 2
2 (1) Posterolateral setae leaf-like *Trombiculindus* Radford, 1948
Type *Trombiculindus squamosus* Radford, 1948
Posterolateral setae normal 3
3 (2) Palpal claw bifurcate, axial prong external (sometimes dorsal) to the smaller, internal (sometimes ventral), accessory prong; always with one or more mastitarsalae III *Eutrombicula* Ewing, 1938
Type *Microthrombidium alfreddugèsi* Oudemans, 1910
Palpal claw either bifurcate, or trifurcate; when bifurcate, the axial prong is internal (or ventral) to the accessory prong; presence of mastitarsalae III variable 4
4 (3) Scutum roughly rectangular 5
Scutum roughly pentagonal 6
5 (4) Galeal seta feathered, setae on palpal femur and genu nude, no whip-like seta on leg III *Leptotrombidium* Nagayo, *et al.*, 1916
Type *Trombidium akamuski* Brumpt, 1910
Without this combination of characters *Trombicula* sensu lato
(Species for which no subgenera have been proposed).
6 (4) Without whip-like setae on leg III or with more than one seta on coxa III *Trombicula* sensu lato
(Species for which no subgenera have been proposed).
With one or more whip-like setae on leg III and a single seta on coxa III 7
7 (6) Feathered setae on leg III subequal, mastifemoralae and mastitibialae absent; (usually parasitic on water-birds) (adults with eyes) *Blankaartia* Oudemans, 1911
Type *Trombidium niloticum* Trägårdh, 1905*
Mastifemoralae and/or mastitibialae present or elongated, feathered setae in place of the whip-like setae; (usually parasitic on terrestrial animals) (adults without eyes) *Neotrombicula* Hirst, 1925
Type *Acarus autumnalis* Shaw, 1790

Endotrombicula.

Lawrence (1949) has discovered a remarkable species on a South African frog and has made it the type of a new genus, *Phrynacarus.* It is however very similar to *Endotrombicula penetrans* Ewing, 1931 also found on African frogs. *Phrynacarus* differs from specimens of *Endotrombicula* (but not the description) only in that the former has four spurs on tarsus I while the latter has a single spur.

1 Four spurs on tarsus I *Phrynacarus* Lawrence, 1949
Type *Phrynacarus fitzsimonsi* Lawrence, 1949
One spur on tarsus I *Endotrombicula* Ewing, 1931
Type *Endotrombicula penetrans* Ewing, 1931

Acomatacarus.

Lawrence (1949) has described three new genera that belong to the Leeuwenhoekiinae. All differ from *Acomatacarus* in that they lack the stigmata and tracheae, but as Lawrence demonstrates, the presence or absence of stigmata is not as fundamental a difference as it was once considered. Other minor differences were also noted. Until further studies have been made it seems desirable to treat Lawrence's genera as subgenera in order to avoid future nomenclatorial confusion.

1 Stigmata and tracheae present *Acomatacarus* Ewing, 1942
Type *Acomatacarus arizonensis* Ewing, 1942
Stigmata and tracheae absent 2
2 (1) Cheliceral blade with ventral teeth but no dorsal teeth *Hyracarus* Lawrence, 1949
Type *Hyracarus typicus* Lawrence, 1949
Cheliceral blade with dorsal and ventral teeth 3
3 (2) Posterolateral scutal setae greatly expanded, ramiform *Austrombicula* Lawrence, 1949

* *Blankaartia* Oudemans, 1911 was based on larvae misidentified as *T. niloticum* but its type designation must stand. *Trägårdhula* Berlese, *Pentagonella* Thor and *Megatrombicula* Michener are regarded by us as synonyms of *Blankaartia.*

Type *Leeuwenhoekia womersleyi* Lawrence, 1949
Posterolateral scutal setae not unusually modified *Austracarus* Lawrence, 1949
Type *Austracarus procaviae* Lawrence, 1949

Leeuwenhoekia.

Comatacarus Ewing, 1942 differs from *Leeuwenhoekia* Oudemans, 1911 in that the former lacks stigmata and tracheae as well as papillae for support of the dorsal setae. These differences are considered here of only subgeneric significance.

1 Stigmata and tracheae present *Leeuwenhoekia* Oudemans, 1911
Type *Heterothrombidium verduni* Oudemans, 1910
Stigmata and tracheae absent *Comatacarus* Ewing, 1942
Type *Comatacarus americanus* Ewing, 1942

Key to the Genera of the Trombiculidae based on Adult Characteristics

Nymphs and adults of trombiculid mites are known for only a few of the genera. Since the nymphs are similar to the adults the following key is based on characteristics that can be applied to either form. This key is presented more to give an idea of how the free-living stages differ in the various genera than to serve as a means of identification. Too little information is available concerning the nymphs and adults to make a suitable classification of the Trombiculidae based on the postlarval stages.

1 Claws on leg I unbranched 2
Claws on leg I trifurcate *Speotrombicula* Ewing, 1946
(Larva unknown) Type *Trombicula trifurca* Ewing, 1933
2 (1) Body with a distinct constriction behind the first two pairs of legs 3
Body without such a constriction *Acomatacarus* Ewing, 1942
(Nymphs described by Womersley, 1945)
Chatia Brennan, 1946
(Nymphs described by Brennan, in press)
3 (2) With setae on the tectum 4
Without setae on the tectum *Walchia Ewing,* 1931
(Observation by G. W. Wharton)
4 (3) With a single tectal seta or tectal setae in tandom 5
With a pair of tectal setae *Hannemania* Oudemans, 1911
(Observation by G. W. Wharton)
5 (4) With a single type of setae on the body. All other genera known as nymphs or adults: *Trombicula, Euschöngastia, Schöngastia* and *Guntherana.*
With long and short setae interspersed *Neoschöngastia* Ewing ,1929
(Nymphs described by Wharton and Hardcastle, 1946)

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ON THE FREQUENCY DISTRIBUTION OF PARASITIC HELMINTHS IN THEIR NATURALLY INFECTED HOSTS

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Parasitologists or public health workers, in making a survey of certain parasites, usually report only the percentage incidence of the parasites. Careful investigators occasionally give also the intensity of infection—the mean number of the parasites found in the hosts. However, if the state of the infection by a given parasite is to be more completely and perfectly understood, the knowledge merely of its incidence and of the intensity of infection is evidently not enough. One should, in our opinion, study in detail the frequency distribution of the parasites, that is, the central tendency of the infection of the parasites in their hosts, the degree of variation of infection of the parasites from their norm, and the direction of divergence of the infection of the parasites. For measuring the central tendency of the infection of the parasites, it is necessary, besides finding out the mean number of the parasites, to investigate also the median value of the infection—a value so selected that greater and smaller values occur with equal frequency, and the mode, a value at which the highest frequency of cases occurs. The state of infection of a parasite will be, in this way, able to be represented in a frequency curve, and if the curve is subsequently fitted, the frequency distribution of the infection of a parasite in a given locality and in a given host can also be calculated.

Realizing the importance of the knowledge of the frequency distribution of the parasites in their hosts, and recognizing that up to the present little work has been done in this respect, we have undertaken a study, with regard to this problem, of 15 species of parasitic nematodes, 3 species of cestodes and 2 species of trematodes in their naturally infected hosts, and have entertained the hope that the present study, although it is only a preliminary report, may reveal certain general but unmentioned phenomena of parasitic infection.

MATERIAL, METHOD AND PROCEDURE

The 15 species of parasitic nematodes used for the present study are *Rhabdias* sp., *Ancylostoma duodenale, A. caninum, Oswaldocruzia peipingensis, Enterobius vermicularis, Syphacia obvelata, Aspiculuris tetraptera, Heterakis spumosa, Heterakis gallinae, Ascaridia galli, Ascaris lumbricoides, Cheilospirura hamulosa, Spiroxys japonicus, Dirofilaria immitis* and *Trichuris trichiura.* They belong to 8 superfamilies: Rhabditidoidea, Strongyloidea, Trichostrongyloidea, Oxyuroidea, Ascaridoidea, Spiruroidea, Filarioidea, and Trichuroidea. The three species of cestodes are *Baerietta baeri, Dipylidium caninum,* and *Cysticercus fasciolaris* and the two species of trematodes are *Echinochasmus perfoliatus* and *Pleurogenes* sp. All of them were collected in Peiping from naturally infected hosts. *Ancylostoma duodenale, Enterobius vermicularis, Ascaris lumbricoides* and *Trichuris trichiura* were obtained from men; *Ancylostoma caninum, Dirofilaria immitis, Dipylidium caninum,* and *Echinochasmus perfoliatus* from dogs; *Heterakis spumosa* and *Cysti-*

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cercus fasciolaris from rats; *Syphacia obvelata* and *Aspiculuris tetraptera* from white mice; *Heterakis gallinae, Ascaridia galli* and *Cheilospirura hamulosa* from chicken; *Rhabdias* sp., *Oswaldocruzia peipingensis, Baerietta baeri* and *Pleurogenes* sp. from toads (*Bufo bufo gargarizans*); and *Spiroxys japonicus* from frogs (*Rana nigromaculata*). The parasites from men, dogs, rats, chicken and frogs were obtained in all seasons, whereas those from mice only in May and June. As to the parasites of toads, *Oswaldocruzia peipingensis* was collected in all seasons and *Rhabdias* sp., *Baerietta baeri* and *Pleurogenes* sp. only in October and November. The majority of the hosts from which the parasites were obtained were adult. Except in the case of men, from whom the worms were collected at autopsy in the Peiping Union Medical College Hospital, all hosts were killed in the laboratory for the purpose of collecting the desired worms. Special attention has been paid to prevent the loss of worms during the time of examination. For instance, when a dog was examined for *Dirofilaria immitis,* not only the heart of the dog was examined, but, in addition, the lungs and the large blood vessels in the vicinity of the heart were also carefully inspected. In the case of nematodes, individuals of the larval stage were not included in the present study, but young worms in which the sexes could be differentiated were counted. If any lot of the nematodes or trematodes collected from a host contained some fragmented specimens of undetermined individuals, the whole lot of worms from that host was discarded. The number of cestodes was counted by the number of scoleces, for which a careful search was made.

RESULTS OF EXAMINATION

The results of the present study on the frequency distribution of parasitic helminths in their naturally infected hosts are given in Table 1 and Graphs 1–20. It will be seen that the frequency curves of all the helminths, although their incidence, intensity of infection and range of infection are more or less different in different species, belong evidently to the category of positive skewness and most of the curves, if fitted, probably belong to the I_j of the Pearsonian frequency curves.

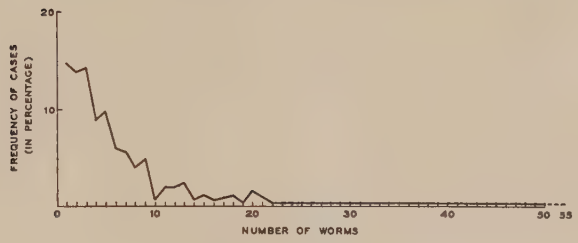
In the case of nematodes, except *Rhabdias* sp., which is parthenogenetic in its parasitic form, all the other 14 species are sexually separated. The frequency curves of both sexes, if made separately, belong also to the same type of curve, as both the sexes have been considered together (see Graphs 21–22). It is interesting to note that in *Heterakis spumosa,* the only helminth in the present study which has a different type of frequency distribution when both sexes are considered together, its frequency curves of a single sex, male or female alone, nevertheless, still appertain to the general type (Graph 23).

CONCLUSION AND DISCUSSION

The present study shows, as mentioned above, that the frequency distribution of parasitic helminths in their naturally infected hosts can, in most cases, probably be well represented by the type I_j of the Pearsonian frequency curve, although their range, incidence and intensity of infection, to a certain extent, are more or less different. Accordingly, the most frequent numbers of the worms in their naturally infected hosts are the lowest numbers, especially of one or two worms. The higher the number of the worms, the less is the frequency. On the other hand, it will be seen from the comparative study of the frequency curves of the 20 species of helminths

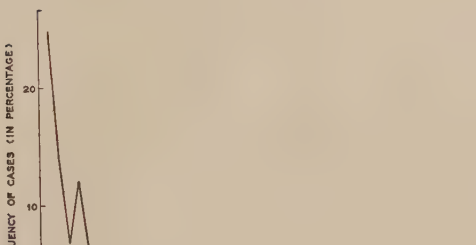
TABLE 1.—*Results of the Present Study on the Frequency Distribution of the Parasitic Helminths in Their Naturally Infected Hosts*

Name of parasites	No. of cases examined	No. of positive cases	Incidence	No. of worms	Range	Mean	Median	1st quartile	3rd quartile
Nematoda									
Rhabdias sp.	316	246	77.9	1,637	54	6.7	3.8	1.8	7.5
Ancylostoma duodenale	888	128	14.4	836	79	6.5	1.8	0.7	6.3
Ancylostoma caninum	202	97	48.0	678	43	7.0	3.2	1.0	8.8
Oswaldocruzia peipingensis	162	101	62.4	1,236	73	12.2	4.0	1.2	14.5
Enterobius vermicularis	1,244	168	13.5	1,974	574	11.8	2.5	0.9	8.1
Syphacia obvelata	150	102	68.0	4,064	214	39.8	22.5	4.9	59.6
Aspiculuris tetraptera	134	100	74.6	7,599	534	76.0	22.3	2.9	93.8
Heterakis spumosa	165	100	60.6	1,583	73	15.8	9.8	2.8	
Heterakis gallinae	183	102	55.7	1,928	258	18.9	5.4	1.7	18.6
Ascaridia galli	201	118	58.7	2,920	292	24.7	7.9	3.6	23.1
Ascaris lumbricoides	1,184	450	38.0	3,835	1,977	8.5	1.7	0.7	3.9
Cheilospirura hamulosa	254	107	42.1	886	80	8.3	4.4	1.7	11.0
Spiroxys japonicus	688	85	12.4	921	108	10.8	3.3	0.9	13.3
Dirofilaria immitis	697	362	51.9	1,883	40	5.2	2.9	1.1	6.5
Trichuris trichura	709	56	7.9	131	31	2.3	0.7	0.4	1.4
Cestoda									
Baerietta baeri	316	49	15.5	180	16	3.7	2.5	0.8	3.8
Dipylidium caninum	101	64	63.4	624	114	9.8	2.9	0.9	6.0
Cysticercus fasciolaris	358	165	46.1	365	20	2.2	0.8	0.4	1.7
Trematoda									
Echinochasmus perfoliatua	101	71	70.3	11,601	3,829	163.4	19.3	4.4	86.3
Pleurogenes sp.	316	42	13.3	388	138	9.2	1.5	0.6	5.8



GRAPH 1. RHABDIAS SP.

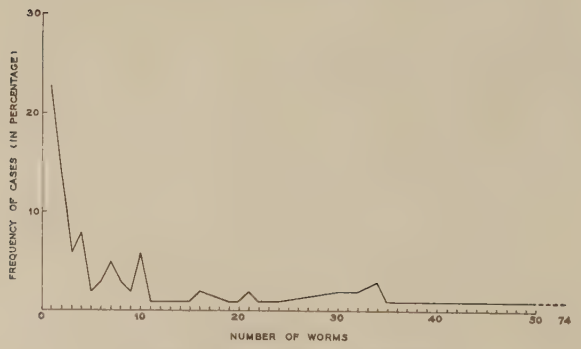
GRAPH 2. ANCYLOSTOMA DUODENALE



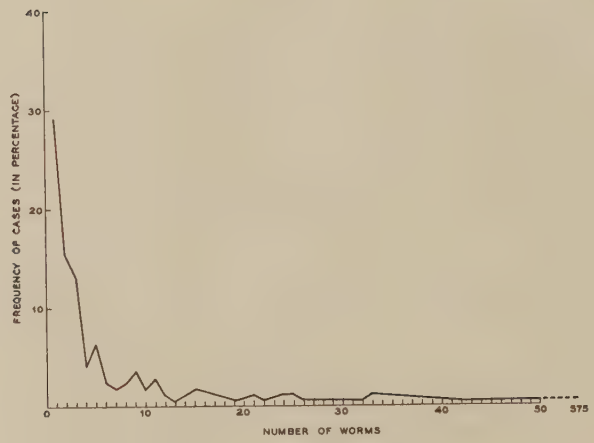
GRAPH 3. ANCYLOSTOMA CANINUM

given in the present paper that, although the frequency distribution of nearly all the species studied exhibits a same type of distribution, different species still differ to a certain degree in the central concentration, dispersion and skewness.

It will be interesting to investigate the difference, if any, of the frequency distribution of a given species of parasite in various conditions, such as in different



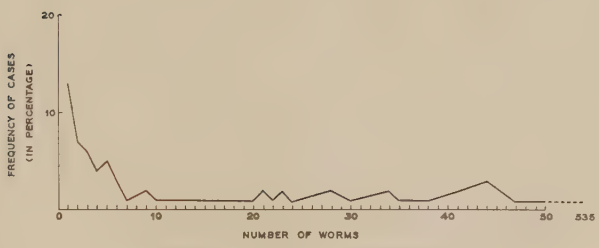
GRAPH 4. OSWALDOCRUZIA PEIPINGENSIS



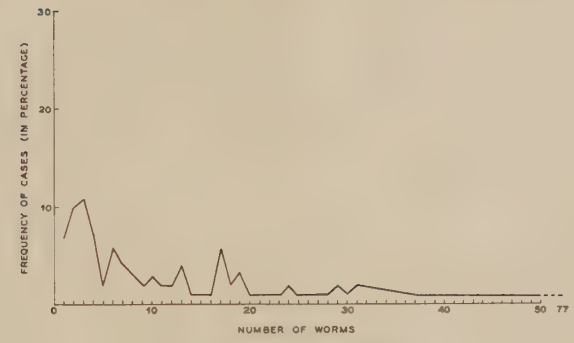
GRAPH 5. ENTEROBIUS VERMICULARIS

GRAPH 6. SYPHACIA OBVELATA

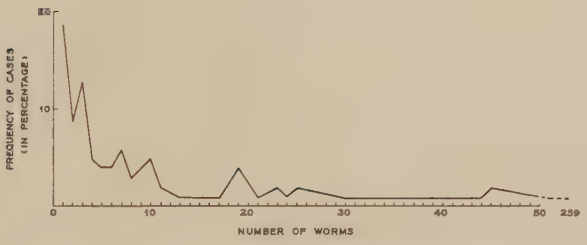
species of hosts, in different ages of hosts, in different duration of infections, and in different localities or seasons of infections. As all these different factors are known to influence the incidence and intensity of the parasitic infection, their frequency distribution under different conditions will most probably also differ to a certain extent. For the study of these biological phenomena, a more extensive examination



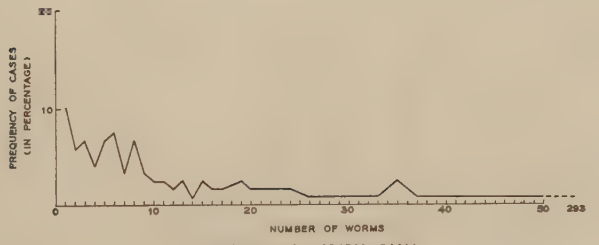
GRAPH 7. ASPICULURIS TETRAPTERA



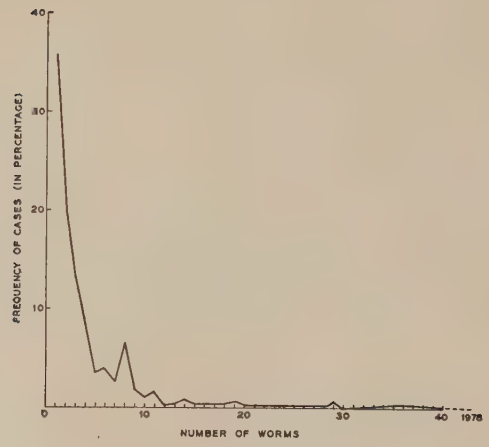
GRAPH 8. HETERAKIS SPUMOSA



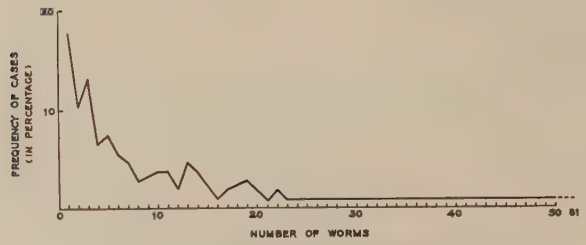
GRAPH 9. HETERAKIS GALLINAE



GRAPH 10. ASCARIDIA GALLI



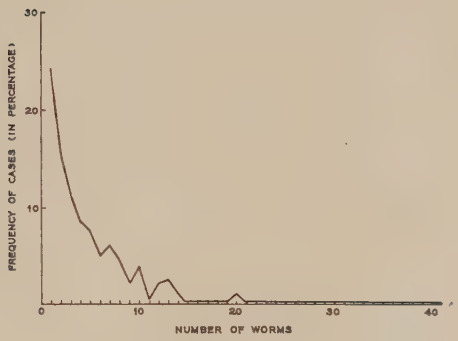
GRAPH 11 ASCARIS LUMBRICOIDES



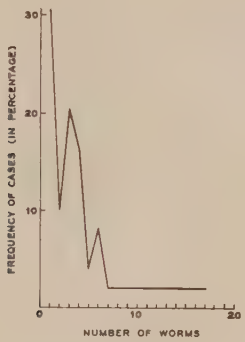
GRAPH 12. CHEILOSPIRULA HAMULOSA

GRAPH 13. SPIROXYS JAPONICUS

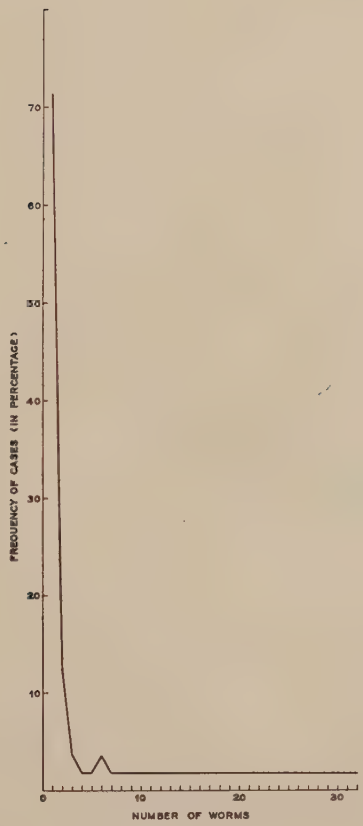
of parasites in their naturally infected hosts as well as in their carefully planned experimental hosts are necessary.



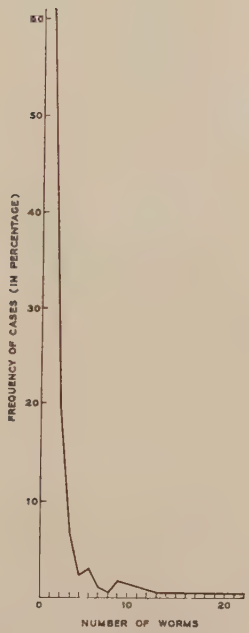
GRAPH 14. DIROFILARIA IMMITIS



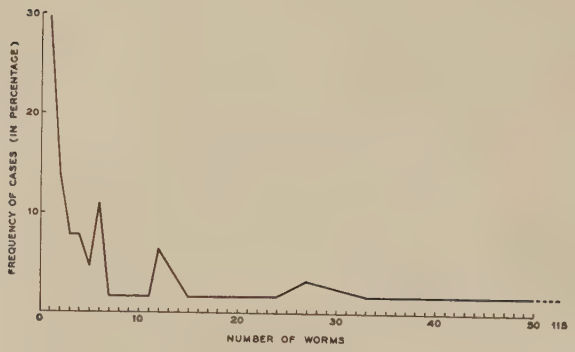
GRAPH 16. BAERIETTA BAERI



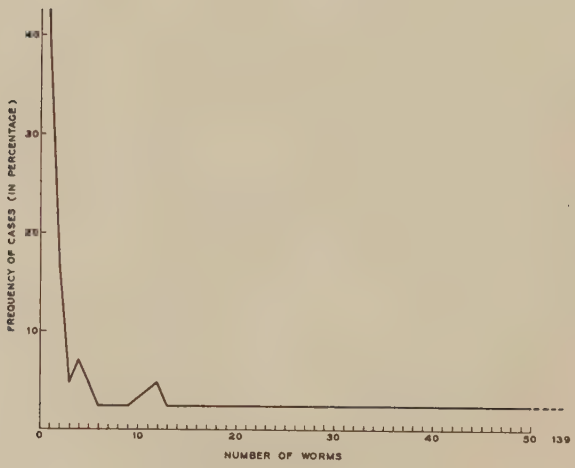
GRAPH 15. TRICHURIS TRICHIURA



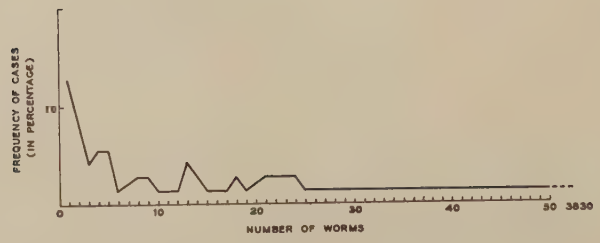
GRAPH 18. CYSTICERCUS FASCIOLARIS



GRAPH 17. DIPYLIDIUM CANINUM

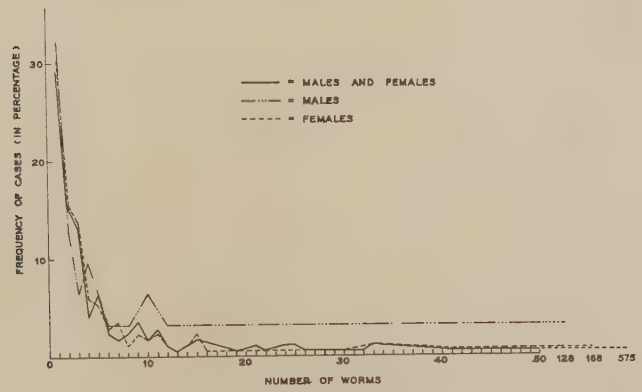


GRAPH 20. PLEUROGENES SP.

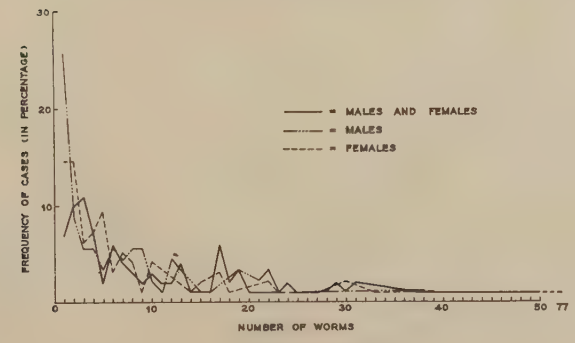


GRAPH 19. ECHINOCHASMUS PERFOLIATUS

GRAPH 21. ASCARIS LUMBRICOIDES



GRAPH 22. ENTEROBIUS VERMICULARIS



GRAPH 23. HETERAKIS SPUMOSA

THE FREQUENCY OF BISEXUAL INFECTIONS OF *SCHISTOSOMA MANSONI* IN SNAILS OF THE SPECIES *AUSTRALORBIS GLABRATUS* (SAY)*

M. A. Stirewalt[1]

INTRODUCTION

It has been generally reported that one snail usually harbors a unisexual infection of *Schistosoma mansoni*. This belief rests on the theory first expressed by Cort in 1921 that "sex in the schistosomes is differentiated in the miracidium stage, and that all cercariae which develop from a single miracidium are of the same sex." This has been consistently substantiated by many investigators who have found that snails infected by a single miracidium always yield cercariae which mature in mice as schistosomes of one sex only. The "hermaphroditic males" described by Vogel (1947) in guinea pigs, rabbits, and hamsters were not found to function as mature females since they possessed only rudimentary female organs and were always recognizable as male worms.

On the other hand, the assumption that only one sex of cercariae is derived from a single snail without reference to the conditions of its exposure is not well founded. Records of small numbers of bisexual infections, both naturally and experimentally incurred, in the vectors of both *S. japonicum* and *S. mansoni* may be cited (Severinghaus, 1928; Vogel, 1941; Mayer and Pifano, 1942; Jaffe, Mayer and Pifano, 1945). In spite of these reports, it was stated in 1945 by the last-mentioned authors that no matter how many miracidia penetrate a snail host only one will develop sufficiently to produce cercariae. This has been refuted recently by Maldonado and Herrera (1949) in naturally infected snails.

In view of the controversial status of this question, it is important for experimental purposes to measure the frequency of bisexual infections in definitive hosts exposed to cercariae from single snails. It is the purpose of this investigation to determine (a) whether laboratory-reared and infected *Australorbis glabratus* exposed to more than one miracidium regularly harbor schistosomes of both sexes as shown by the recovery of adult worms from infected mice; and, if so, (b) what the proportion is between all male, all female, and bisexual infections in snails from multiple-miracidial exposures.

MATERIALS AND METHODS

The strain of *S. mansoni* used in this study was from Puerto Rico. It had been maintained in this laboratory for more than two years in mice or hamsters and in snails of the species *A. glabratus* (Say), also from Puerto Rico.

An inbred strain of white mice, the Webster line of the Carworth Farms-Webster mice, obtained from the National Institutes of Health, was used, since previous ex-

* Naval Medical Research Institute, Bethesda, Maryland. The opinions contained in this paper are those of the author. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

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periments have proved these mice to be suitable hosts for *S. mansoni.* Animals weighing 14 to 20 grams were employed at exposure.

Miracidia were obtained from ova in finely minced livers of freshly killed hamsters with infections of 8 to 12 weeks' duration. The minced liver was washed several times in distilled water, placed in gum evaporating dishes in 80 ml. of distilled water, and allowed to stand in a small well-lighted oven at 35° C. until the swimming miracidia could be observed at the surface.

With the aid of a dissecting microscope, the miracidia were picked out one at a time in a small pipette and placed in 8-ml. antigen vials in a small drop of water. Each vial was checked to make certain that the desired number of actively swimming miracidia was present. Just enough distilled water was added to cover a small snail, which was immediately placed in the vial. Snails were exposed for about 18 hours at a room temperature of 23° C. to seven miracidial densities: 1, 2, 3, 4, 5, 10,* and 20.* At the end of the exposure period, the snails were removed to culture dishes according to the number of miracidia to which they had been exposed. Snails were maintained routinely in dechlorinated tap water and fed dried maple leaves and lettuce.

After 28 days, the snails were placed singly in antigen vials in five ml. of dechlorinated tap water at room temperature from 9: 30 a.m. to 2: 30 p.m. daily, the water in each vial examined for the presence of cercariae, and the infected snails isolated from those not liberating cercariae. To insure complete recognition of infected snails, those which had previously not shown signs of infection were re-examined twice weekly for ten weeks. From two to 13 weeks after the beginning of cercarial emergence, mice were exposed in the following manner. Cercariae from one snail were poured into three to five small finger bowls, approximately 100 to 200 cercariae per dish; or, if it was desired to use known numbers of larvae for infection, 100 cercariae were counted onto the bottom of each dish. In the latter procedure, the pipette was thoroughly washed in boiling water, air dried, and examined under a dissecting microscope to prevent the transfer of cercariae from one vial to another. One mouse was placed in each infecting dish and dechlorinated tap water added to a depth of one cm. The dishes containing cercariae from one snail were covered with a single wire screen which in turn was covered with gauze. One group was placed far enough from the next to prevent contamination by the splashing of water containing cercariae from another snail.

Two hours were allowed for cercarial penetration. At the end of this time the mice which had been exposed to cercariae from one snail were placed together in one cage. Seven or eight weeks later they were killed with chloroform and the schistosomes dissected from their veins. If the cercariae had been counted, the number as well as the sex of the worms was recorded.

RESULTS AND DISCUSSION

The sex of infections produced in 629 snails from seven different series of exposures to miracidia of *S. mansoni* is given in Table 1. Only unisexual infections were observed in snails infected by single larvae. Multiple-miracidial snail exposures, on the other hand, even some of those employing only two per snail, pro-

[2] ±2 miracidia. The difficulty of counting free-swimming miracidia in these numbers in a small vial may have introduced a small error.

vided cercariae which matured in mice as adult parasites of both sexes. Thirty-eight of the 50 infections in snails which had been exposed to 20 miracidia were bisexual.

Obviously, the assumption that only one miracidium will develop to the stage of cercarial production in *A. glabratus* (Jaffe, Mayer, and Pifano, 1945) is unwarranted, as was shown in naturally-infected snails by Maldonado and Herrera (1949). As is indicated by the proportion of bisexual infections in Table 1, at least 28 per cent of the "two-miracidial" snails supported the development of more than one primary sporocyst, 27 per cent of the "three-miracidial" infections, etc. Undoubtedly also, some of the single sex infections were produced by multiple miracidial penetrations and the development of several primary sporocysts of the same sex.

TABLE 1.—*Sex of* S. mansoni *infections in snails exposed to varying numbers of miracidia*

Number of miracidia	Number of snails	Number of infections		
		Male	Female	Bisexual
1	79	45	34	0
2	100	39	33	28
3	100	36	37	27
4	100	38	27	35
5	100	26	30	44
10	100	29	19	52
20	50	8	4	38

On the other hand, infection of all the snails did not always occur; many exposed to active miracidia under favorable conditions failed to show any sign of infection (Table 2). Even exposures of 10 and 20 miracidia per snail did not produce infections in all the snails.

These figures are not in good agreement with those of Schreiber and Schubert (1949) who exposed snails of the same species to 1, 3, 7, and 10 miracidia of *S. mansoni* and recorded infectivity rates of 14, 55, 70, and 85 per cent respectively, or with the reports of complete infection of all snails exposed communicated personally by several other investigators. Since the rate of infection from similar exposures in this laboratory of different groups of snails to equal numbers of miracidia has not always been consistent, it may be that these disagreements are related to differential snail susceptibilities to the schistosome strain employed.

TABLE 2.—*Percentage of snails infected from varying miracidial densities*

Number of miracidia	Number of snails exposed	Groups of snails	Per cent of snails		
			infected	uninfected	dying*
1	844	4	32.5	57.3	10.2
2	360	8	36.7	53.0	10.3
3	340	9	47.6	37.4	15.0
4	266	8	50.3	29.0	20.7
5	298	8	50.4	25.8	23.8
10	176	7	72.7	9.1	18.2
20	104	5	70.2	4.8	25.0

* Dying prepatently.

However, other inferences than that some snails are highly resistant to infection may be drawn from the data. Two independent series of events must be considered in the production of infection in a snail: the miracidium must find and enter the snail, and development from miracidium to cercariae must follow. While it has been generally considered that an "attraction" of snails for miracidia influences the behavior

of the larval parasite during the first events, an inhibition by the snail of the subsequent development of sporocysts has been postulated.

If it is assumed that these snails attract the miracidia, then under the conditions of laboratory exposure most of the miracidia present in the small volume of water used would penetrate. If male and female miracidia are present in about equal numbers, a logical assumption from these studies, then, since at least one sporocyst of each sex must mature to produce a bisexual infection, the percentage of bisexual infections should be that given in Table 3. Comparison of the observed percentages of bisexual infections with those calculated shows that such maximal penetration and development did not occur in the susceptible snails. Either all the miracidia in a favorable position to enter the snails did not do so, or many which penetrated did not develop. It is impossible at present to give proper emphasis to these factors, but it is hoped that further studies may solve these problems. Observations in this laboratory of miracidial behavior in the presence of the snails, however, support the contention that there is no "attraction" of the molluscs for these miracidia. It is therefore suggested that the contact of the latter with the soft penetrable tissues of a snail may be a chance phenomenon. In addition, probabilistic evidence for the independence of miracidial behavior in the infection of these snails is in preparation from these data.

TABLE 3.—*Probabilities of male or female ($P_{m \text{ or } f}$) and bisexual (P_b) infections together with the observed percentages of bisexual infections for the miracidial densities given*

Miracidia	$P_{m \text{ or } f}$	P_b	Observed percentage$_b$
1	1.00	.00	.00
2	.50	.50	.28
3	.25	.75	.27
4	.125	.875	.35
5	.0625	.9375	.44
10	.0018	.9982	.52
20	....	....	.76

Discussion of several possible reasons for the widely-accepted hypothesis of single sex infections in individual snails may be of value here. It is probable that snails infected naturally in waters in which the schistosomes are endemic often do harbor only one sex of the parasite. As has been suggested (Vogel, 1941), climatic conditions may affect male and female juvenile parasites differently; or, under natural conditions, the density of the miracidia may be so low that only one may be in a position to infect a snail. Further study of the sex of naturally incurred snail infections should be made.

Other factors which might also tend to limit schistosome infections to one sex in snails were considered: the size of the snail, the space and food available to the parasite. If such conditions as these do influence the number of bisexual infections in snails, then more bisexual infections would be observed in the early days of the patency of the infection than later. This would mean that a newly-infected mollusc might support the development of several primary sporocysts, but that as the infection progresses, it might be reduced to the progeny developing from only one sporocyst. The experimental findings, however, were not compatible with this suggestion. Of 12 snails with bisexual infections which were followed from the early days of their patency, all continued to provide cercariae of both sexes until their deaths, as long

as three months later. Conversely, snails with early unisexual infections were never found to show evidence later of a bisexual infection.

Several facts of interest were derived from examination of the worms recovered from mice which had been exposed to known numbers of cercariae. It was not possible to deduce the comparative numbers of male and female miracidia producing the bisexual infections by an analysis of the relative numbers of adult male and female schistosomes recovered from mice. However, it was found that all of the mice infected with cercariae from one bisexually-infected snail were strikingly consistent both in the total number of worms they harbored, and in the proportion of one sex of worms to the other, the latter remaining constant even when the cercariae were derived from the snails at different periods during the progress of the infection. In bisexual infections produced by two miracidia, a preponderance of one sex over the other was interpreted as reflecting a disproportion either in the productivity of the sporocysts or in the infectivity of cercariae from different sporocysts.

The average number of adult schistosomes per definitive host series, i.e., from cercariae from snail exposures to one miracidium, two miracidia, etc., was found to vary greatly, and without reference to the density of miracidia at the snail exposures. An average of nine worms per mouse was recorded in the "10-miracidial" series; 22 worms per mouse were recovered in the "one and 20-miracidial" series. The average standard deviation in the parasite loads of the mice within a series was seven. Such a wide variation is believed attributable, not to differences in cercariae related to the number of miracidia to which the snails were exposed, but to the fact that some of the cercariae employed for definitive host infections were derived from the snails during the early weeks of the patency of their infections when excessive variability in cercarial infectivity may be expected (Evans and Stirewalt, 1949). Had all the cercariae been recovered after a more stable snail-parasite balance had been established, less over-all variation in numbers of worms per mouse might have been expected.

SUMMARY AND CONCLUSIONS

The sex of infections of *Schistosoma mansoni* developing in *Australorbis glabratus* from exposures of individual snails to 1, 2, 3, 4, 5, 10, and 20 miracidia each has been observed upon the recovery of the schistosomes maturing in mice from the cercariae from these snails.

1. Many individual *A. glabratus,* perhaps of a resistant genetic strain, were found to be refractory to this strain of *S. mansoni.*

2. Unisexual infections of schistosomes resulted from exposures of one miracidium per snail; bisexual infections from 2, 3, 4, 5, and 10 miracidia varied from 28 to 52 per cent of the total infections; while 76 per cent of the infections from 20 miracidia were bisexual.

3. Summated unisexual infections were about equally divided between male and female.

4. The proportion of bisexual infections among susceptible snails, indicating the maturation of at least one miracidia of each sex per snail, was lower than that to be expected on the basis of an "attraction" of the molluscs for the miracidia and the unihibited development of the latter after penetration. It is suggested that per-

haps the contact of these miracidia with the soft tissues of the snail is a chance phenomenon.

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ERYTHROCYTE SEDIMENTATION RATE IN PIGEONS INFECTED WITH *PLASMODIUM RELICTUM**

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The effect of the volume per cent of red cells on the suspension stability of blood has been reported by Rourke and Plass (1929), Jones (1929), Kopp (1942), and others. Rubin and Smith (1927) and Hubbard and Geiger (1928) studied the effects of varying the cell volume content of the blood from both normal and pathological human cases. They were able to show the effect of changing the volume of cells by adding homologous plasma to or by removing plasma from the cells. No foreign substance was introduced. Their work demonstrated beyond doubt the marked effect of the cell concentration on the sedimentation rate. Whittington and Miller (1942) state that rouleaux-formation is responsible for the increase in sedimentation rate and that all variables which influence the sedimentation rate manifest their effect in this manner. According to Suzue (1927) the electric charge on the cells is the factor determining the sedimentation velocity. Very little agreement is found among the many workers who have observed the effects of fibrinogen, globulin, albumin, cholesterol and other substances. An increase in the sedimentation rate of blood in malaria infections has been noted by Radasavljevic and Ristic (1926), Paterni (1928), Colucci (1929), Kehar and Harbhagwan (1937), and Greig and Neil (1939). The increased rate was correlated with the intensity of the infection but no one factor was given as being of outstanding significance.

Very few reports on the sedimentation rate deal with bird blood. Since rouleaux formation and normal aggregation so common in mammalian blood are rare in avian blood, determinations of the sedimentation rate without the influence of these factors are more easily made in the latter. Numerous determinations have been made on more than 100 pigeons both normal and infected with *Plasmodium relictum* Grassi and Feletti. The results of this work indicate that the most important factor influencing the sedimentation velocity of red blood cells in plasma from either normal or infected birds is the volume per cent of cells present in the blood.

METHODS

These determinations were made on the blood of white Carneaux pigeons. The malarial parasite used for the infections was the 1P strain of *Plasmodium relictum,* infections being obtained by intravenous inoculation with blood from a pigeon previously infected.

A very small amount (1 or 2 drops per ml. of blood) of a 1% solution of heparin in 0.9% sodium chloride was used as anticoagulant.

In experiments involving different cell volume percentages, or in which exchanges of cells and plasma were made, the required amount of blood for each determination was centrifuged for separation of cells from plasma. The proper concentrations

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or dilutions, and the desired exchanges were then made. Tests were run which demonstrated that this brief centrifuging and separation of the blood components did not affect the sedimentation of the cells. The Wintrobe hematocrit tube was used and readings made to the closest approximation of one tenth millimeter by the use of a hand lens.

Readings were taken at the end of 5 minutes, 15 minutes, 30 minutes, and one hour and at intervals of one-half or one hour for the succeeding 4 or 5 hours. The results given are those recorded for the first hour of settling beginning after the first five minutes, since it was found that this period gave the most uniform and reliable measurements. At the end of the observation period, each tube was centrifuged at 2000 revolutions per minute for one hour, and the hematocrit recorded.

RESULTS

The average erythrocyte sedimentation velocity of whole blood of pigeons which have developed heavy infections of malaria is about 3 times as rapid as the average velocity of the red cells of whole blood of normal pigeons. The sedimentation rate ranges from 0.4 mm. to 1.2 mm. per hour for the whole blood of normal birds, and from 1.5 mm. to 3.5 mm. per hour for the whole blood of infected birds (Fig. 1). A series of determinations was made on blood obtained at various stages in the progression of the infection in 6 birds. Daily parasite counts were made and the volume per cent of cells was obtained for each blood sample on which the sedimentation rate was determined. It soon became apparent that the increase in the rate of settling was correlated with the cell volume per cent. During the early part of the infection, as the parasites are increasing rapidly in number, the rate varies only slightly from the normal. As the number of erythrocytes is reduced as a result of the infection the sedimentation rate increased and, following the disappearance of the parasites from the blood stream the rate decreased as the cell count returned to normal. Results of determinations on one bird illustrate the inverse correlation of the sedimentation rate with the parasitemia. On the second, the ninth and the thirteenth days of infection the sedimentation rates were 0.5 mm., 1.7 mm. and 0.9 mm. per hour respectively. On the same days the parasite counts were 100, 3180 and 0 per 10,000 R.B.C. It was not feasible to remove blood from a single infection on several succeeding days as this in itself would materially alter the blood picture.

TABLE 1.—*Mean sedimentation rates of R.B.C. at various cell volume percentages. In mm/hr.*

Cells	Plasma	% Cell Vol. 43–48	33–38	25–30
Normal	Normal	0.83 (12)*	1.44 (9)	2.69 (7)
Range		(0.56–1.10)	(0.87–2.07)	(1.86–3.80)
Infected	Infected	0.78 (11)	1.23 (26)	2.39 (10)
Range		(0.40–1.31)	(0.66–2.18)	(1.85–3.16)
Infected	Normal	0.83 (7)	1.49 (9)	1.99 (5)
Range		(0.43–1.10)	(1.10–2.40)	(1.46–2.50)
Normal	Infected	0.68 (11)	1.51 (6)	2.53 (4)
Range		(0.21–0.98)	(0.98–1.96)	(1.85–3.90)

* Number of readings on which each mean rate is based.

Following the observation of the increase in sedimentation rate of infected blood, attempts were made to determine the factor, or factors, present in either the plasma or the cells, responsible for the increased rate. Exchanges of plasma were made on equal amounts of blood from a normal pigeon and from an infected pigeon. After

centrifuging 2 ml. amounts of normal and of infected whole blood the plasma was removed from each tube and placed with the cells in the alternate tube. Representative results are shown in Fig. 2. Curves 1 and 2 show the rate for the normal whole blood and for infected whole blood respectively. When the plasma on equal amounts

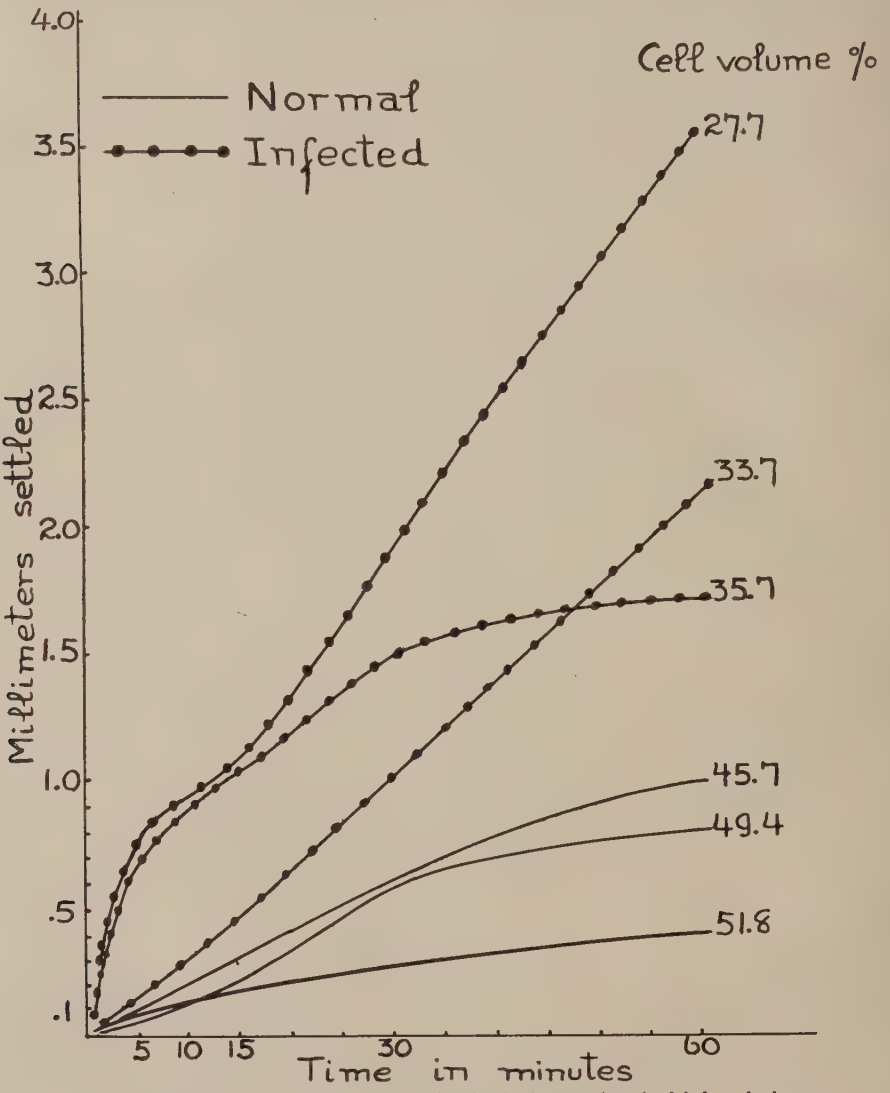

FIG. 1. Erythrocyte sedimentation of whole blood of normal and of infected pigeons.

of these two samples is exchanged the cell volume per cent of the suspension of infected cells in normal plasma is increased and the sedimentation rate is decreased (Curve 4). Correspondingly, the cell volume per cent of the suspension of normal cells in infected plasma is decreased and the sedimentation rate is increased (Curve 3).

In order to determine the effect of the change in cell volume on the rate of settling of the cells the exchanges of plasma were carried out so as to retain the original volume of cells of the whole blood. In Curve 5 (Fig. 2) is shown the sedimentation rate of *infected cells* suspended in *normal plasma* to approximate the concentration of the infected whole blood. In Curve 6 is shown the rate of settling of *normal cells* in *infected plasma* at the cell concentration of normal whole blood. All results shown in Fig. 2 are based on determinations on blood obtained from one normal and one infected pigeon.

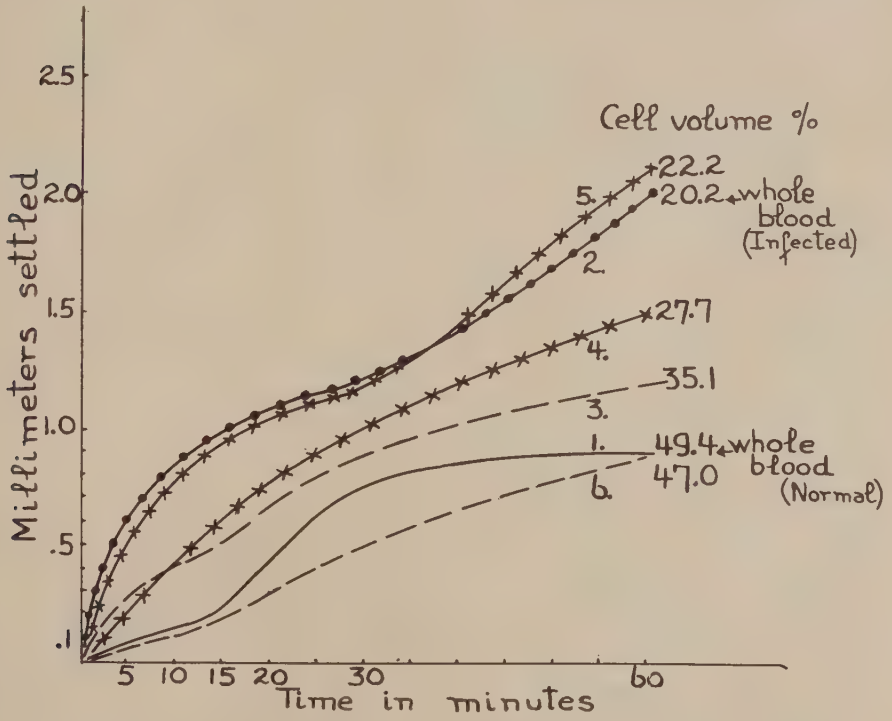

FIG. 2. Effect on sedimentation of exchange of cells and plasma of a normal and of an infected pigeon.

1. Normal whole blood.
2. Infected whole blood.
3 and 6. Normal cells in infected plasma.
4 and 5. Infected cells in normal plasma.

Exchanges of plasma were made on both normal and infected cells at various cell concentrations. In Table 1 are given the averages of the sedimentation rates after exchanges of plasma were made at the specified ranges of cell concentration. If placed on a graph the curves for the individual rates would fall in three fairly distinct groups corresponding to the 3 ranges of concentration of cells. The cells settled at approximately the same rate when the volume per cent was the same regardless of the source of the plasma.

In Fig. 3 are given the results of determinations on *normal blood* and on infected blood at various concentrations. Curve 1 shows the rate of normal whole blood, and

Curves 2 and 3 show the rates of normal whole blood diluted with homologous plasma. Curve 4 shows the rate of the infected whole blood. When this same blood is diluted with its plasma the results shown in Curve 5 are obtained. Concentration of a sample of the same *infected blood* so as to contain 39.7% of cells reduced the sedimentation rate as shown in Curve 6, and concentration to 48.6% reduced the rate still further as shown in Curve 7.

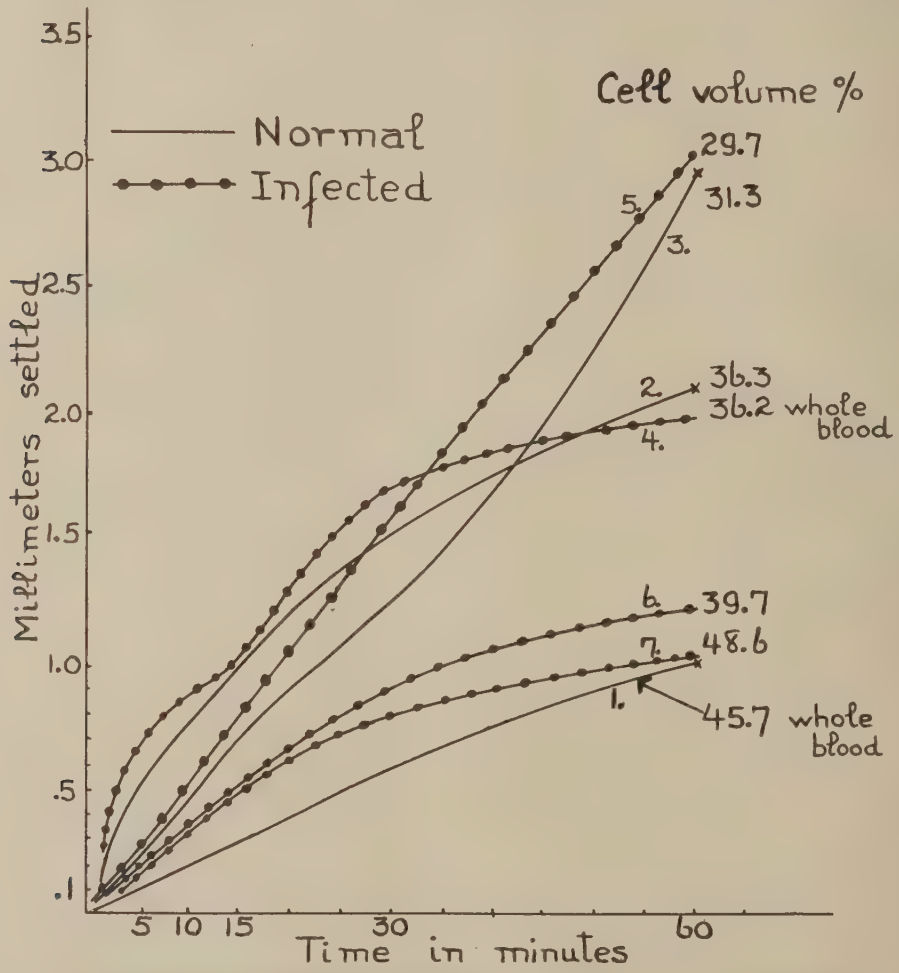

FIG. 3. Erythrocyte sedimentation of normal and of infected blood at comparable cell volumes.

DISCUSSION

In these experiments the use of bird blood having nucleated erythrocytes has made it difficult to compare the results with those obtained by other workers on mammalian blood. Since no rouleaux formation or normal aggregation has been observed in the pigeon blood, one outstanding factor in the rate of settling of mammalian blood is eliminated, and it is therefore possible to evaluate the effect of other factors in its

absence. The aggregation or clumping of cells noted in a few of the tubes, usually after 2 hours of settling, appeared to be abnormal as the rate was drastically increased when these clumps formed. When clumping of cells or other abnormal appearances occurred during the first hour of settling the results were not included in this report.

In attempting to show that the rouleaux-aggregation factor is responsible for variations in the sedimentation rate Whittington and Miller (1942) failed to determine the rates of various percentages of cells in the same plasma. By diluting the plasma with saline citrate solution (apparently hypertonic) other factors evidently were introduced.

During the course of the malaria infection in pigeons the gradual increase in the sedimentation rate to a peak, and the subsequent decrease might conceivably result from variations in the plasma constituents as indicated by Jones (1929), or from changes in the cell surface as found by Suzue (1927). The initial exchanges made on equal amounts of blood from normal and infected birds indicated that some factor was present in the plasma of the infected bird that caused both infected and normal cells to sediment more rapidly (Fig. 2, Curves 2 and 3). However, when plasma and cells are exchanged so as to retain the original cell volume of the whole blood of each bird the results do not indicate the presence of such a factor. These results are more readily explained by the use of a hypothetical example. The plasma obtained from 2 ml. of normal blood with 50% cell volume would be approximately 1 ml. From 2 ml. of infected blood with a cell volume of 25%, 1.5 ml. of plasma would be obtained. When these two amounts of plasma are exchanged, the 2 ml. of normal blood becomes 2.5 ml. with 40% cells. The 1 ml. of normal plasma added to the 0.5 ml. of infected cells would give 1.5 ml. with a 33% cell volume. The 2 blood suspensions which originally had 50% and 25% of cells now become suspensions with 40% and 33% of cells respectively. The altered cell volumes are accompanied by an increase in the sedimentation rate of the normal cells in the infected plasma and a decrease in the sedimentation rate of the infected cells in the normal plasma. The experiments retaining the original percentages of cells but with an exchange of plasma give sedimentation rates comparable to that of the original blood samples. The slight variations found here are no greater than the variations found in normal whole blood. It was difficult to obtain a specified concentration of cells since the blood was centrifuged only sufficiently to cause settling. To have centrifuged the blood more might have introduced other factors into the results. (For specific experimental data see Curves 1, 2, 3, and 4 of Fig. 2.)

The results of numerous determinations on normal whole blood, and normal blood diluted with homologous plasma show that the more concentrated cells settled at a slower rate. When compared with the sedimentation rates of similar cell concentrations of infected blood the similarity of the results is striking (Table 1). For example, infected whole blood with a cell volume of 23% settled at approximately the same rate as normal blood diluted to 23% by the addition of plasma from the same blood. Likewise, concentration of infected blood to the cell volume per cent of normal blood resulted in a sedimentation rate corresponding to that of normal blood of the same cell volume. The cell concentrations of whole blood samples of many birds with light infections were intermediate between the normal and the extremely low values of some of the highly infected birds. The sedimentation rates of these

were intermediate in value. Dilutions of some of these samples to lower cell concentrations produced rates that correspond with the rates of the infected whole blood with similar low cell concentrations (Table 1).

Likewise, all exchanges of plasma and cells at various cell concentrations produced sedimentation rates which correspond closely with those obtained in similarly concentrated or diluted samples from infected or normal pigeons. These results indicate that no factor affecting the sedimentation rate is present in infected blood that is not present in normal blood.

It was found impractical to calculate standard deviations for the mean values given in Table 1 since each column represents a range in cell volume percentages sufficient to cause wide variations in the resulting sedimentation rates. The range of 5% in the cell volume which was used was sufficient to allow an increase (or decrease) of 100% in the rate of settling, particularly in the more dilute samples. In all of the groups there were generally one or two extremes on either side which extended the range.

There is no doubt that other factors such as the protein content of the plasma, the electric charge of the cells, the size and specific gravity of the cells, the temperature and pH of the blood and the type of anticoagulant used may affect the rate of sedimentation of blood. However, most of these factors vary only slightly and their effect is so much less marked than the effect of the cell concentration that they are of relative insignificance. These and other factors appear to affect the rouleaux-aggregation of mammalian cells and in this manner may cause a greater effect on the sedimentation rate of mammalian blood. The results of these experiments seem to indicate that none of these factors work through such a mechanism in bird blood, and consequently, are of much less significance.

SUMMARY

At the peak of destruction of red blood cells during an infection of *P. relictum* in the pigeon the erythrocyte sedimentation rate is increased to approximately 3 times that of normal blood. In the blood of pigeons, in which rouleaux formation and red cell aggregation are negligible, the most important factor responsible for the increased sedimentation rate is the volume per cent of red blood cells. The hematocrit reading of the blood of heavily infected pigeons frequently is reduced to 50% of the normal value. Increase of the red cell count by removal of plasma from infected whole blood caused a decrease of the sedimentation rate. Decrease of the red cell count by addition of homologous plasma to normal whole blood produced an increase in the sedimentation rate. The rates of settling were approximately the same in diluted normal blood, in infected whole blood, in normal cells in infected plasma, and in infected cells in normal plasma whenever the cell volume per cent was the same for the various samples. When infected blood and the various combinations of cells and plasma were concentrated to the cell volume per cent of normal whole blood the sedimentation rates closely approximated that of normal whole blood.

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A SURVEY OF PARASITISM OF THE STARLING *STURNUS VULGARIS* L. IN NORTH AMERICA*

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Scant knowledge of the nature and extent of parasitism as it exists in the starling in North America is available, yet this bird is, unfortunately, extremely common and widespread throughout this continent. Information pertaining to this subject might prove of value in establishing the importance of the starling as a disseminator of parasites among wild and domesticated birds, and as source material for teaching in the field of parasitology. In addition, a comparison of parasitism of the starling in North America with its occurrence in this host in Europe would reveal the possible role that this bird may have played in introducing parasites into North America on its importation in 1890; and at the same time it would demonstrate whether this alien bird has acquired other parasites from native birds since its sojourn here.

An attempt is herewith made to assemble data from previously published work on parasitism of the starling in North America and by a further examination of 300 starlings to give as complete a picture as possible of the parasites of this bird and an indication as to their prevalence in North America. This study, however, has been confined to the ectoparasites and to the parasites of the respiratory-digestive tract of the host, which for brevity's sake will henceforth be referred to as endoparasites.

MATERIAL AND METHODS

The present investigation is based on an examination of 300 starlings. For convenience these have been placed into two divisions—the 'summer' and 'winter' groups. The 'summer' birds comprise 175 individuals of which 149 are immatures (identified by the persistence of bursa fabricii) and 26 adults. The 'summer' birds were shot in New York state, the first eight in July, '44 and the remaining 167 between August 17 and October 11, '45, apparently in the progress of migration. The 'winter' group of 125 birds, of which all but four were adults, was killed between December, '44 and March, '45 and between November 24, '45 and April 27, '46 and came from the following states—New York (9); Massachusetts (41); Connecticut (6); Maryland (3); Ohio (19); and Indiana (47) (Tables 2, 4). A study of the ectoparasites was omitted for the first 13 individuals collected (July 8, '44; Dec. 5, '44–March, '45, New York state).

The examination of all of the birds was done within 24 hours following death of the host with the aid of a binocular microscope and with a strong light projecting on the region under observation. The ectoparasites were found on careful scrutinization

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of both feathers and skin of the bird and subsequently preserved in 70% alcohol. The alimentary-respiratory tract was next removed and the endoparasites obtained by dissecting out the component portions of the tract in warm water in separate dishes. Half of the intestinal contents was centrifuged (sugar flotation method) and examined for PROTOZOA and worm eggs; the remainder was set aside for three days in 2% potassium dichromate solution and subsequently checked for presence of coccidial spores. PLATYHELMINTHES and ACANTHOCEPHALA were fixed in Bouin's fluid following relaxation in the refrigerator; permanent preparations were made later. NEMATHELMINTHES were dropped into hot glycerine-alcohol and studied as temporary mounts in lactophenol. In a few instances sections were prepared of portions of the digestive tract with parasites *in situ* to obtain a microscopic picture of such infestations.

The incidence of parasitism ranks high in *Sturnus vulgaris* L. since in the present investigation all were infested by one or more parasites—95.1% by ectoparasites and 99.0% by endoparasites. Ninety per cent of the 300 hosts harbored helminthes. As there was no significant difference in the numbers of immature and adult birds harboring the ectoparasites these data were omitted in table 2.

TABLE 1.—*Ectoparasites recorded for the starling in North America*

Classification Name of parasite	*	x	In present survey % infection	In present survey Location on host	Origin
INSECTA					
Mallophaga					
Myrsidea cucullaris			—		Old World
Degeeriella nebulosa			72.5	body feathers	Old World
D. illustris			—		America
Menacanthus spinosum			81.5	body feathers	Old World
Diptera					
Ornithomyia fringillina	*		0.3	skin	Old World
Siphonaptera					
Ceratophyllus gallinae		x	1.7	skin	Old World
Epitedia wenmanni			—		America
ARACHNIDA					
Acarina					
Haemaphysalis leporis-palustris		x	—		America
Ixodes brunneus		x	—		America
Atricholaelaps megaventralis			—		America
Liponyssus sylviarum		x		skin, body f.	Old World
Dermanyssus gallinae	*	x	3.8	skin, body f.	Old World
D. prognephilus	*			skin, body f.	America
Trouessartia rosterii	*		60.3	wing feathers	Old World
Rivoltasia sp.	*		0.7	body feathers	?
Cheyletiella sp.	*		5.2	skin, body f.	?

* first published record for the starling in North America.
x parasites also of gallinaceous birds.

ECTOPARASITES

A total of ten ectoparasites has been recorded in published data for the starling in North America. These comprise two ticks, two mites, four lice and two fleas. This total does not include the finding of larvae *Protocalliphora* sp. on nestling starlings (Mason, 1936). The present investigation adds six parasites, five mites and an hippoboscid fly, to the original list, thus bringing the count to sixteen (Table 1).

In addition to determining the incidence and variety of ectoparasites on the starling, some observations were made on the habits of certain of them. For example, it was found that only the blood-sucking ectoparasites tended to leave the host following its death, while the 'feather-mites' and one species of lice remained on the

host's plumage. Furthermore two observations suggest that the starling may use its bill to free its body from vermin. The single bird that was found with a deformed bill was heavily infested with MALLOPHAGA—a condition similar to that noted in a junco by Worth (1940). Secondly, in a few cases, lice were present in the stomach contents, a finding which parallels the report of Fox (1940) on the presence of fleas in the starling's stomach.

INSECTA

MALLOPHAGA: Lice exhibit relatively high host-specificity, and those that parasitize the starling in Europe are *Myrsidea cucullaris Nitzsch, Degeeriella nebulosa* Burmeister, *Menacanthus spinosum* Piaget, and *Philopterus sturni* Schrank (Harrison, 1916; Thompson, 1936). The first three species and also *D. illustris* Kell., a parasite of the purely American family of birds, the ICTERIDAE, have been recorded for the starling in North America (Peters, 1936). Thus the starling on its importation brought with it three of its four species of lice and has since acquired an American louse (Table 1). It has apparently been responsible for the spread of *D. nebulosa* to the Eastern robin, *Turdus m. migratorius* L. (Wilson, 1928).

In the present study, *D. nebulosa* and *M. spinosum* were the only two lice found on the starling and both were located on the body plumage. Both species occurred on hosts from all the states furnishing them, and in three states (Conn., Ind., Mass.) provide new state records. Undoubtedly they are to be found wherever the starling is present. The incidence of mallophagan infestation was high, 93.4%, even higher than that of 85.5% for 76 starlings examined by Geist (1935). New data on their frequency of occurrence proved to be of interest (Table 2). There was a marked reduction in number of infected birds and in number of lice per bird during the winter months (Fig. 1). The percentage of infestation was 68.3 and 71.9 for *D. nebulosa* and *M. spinosum,* respectively, in the 'summer' compared to 25.0 and 35.0 for the 'winter' group. Lice were entirely absent in January and few, predominantly nymphs, were present in February. Two types of eggs could be distinguished amongst the plumage, differing from each other in shape and location. Since eggs of different species of MALLOPHAGA have not as yet been described, it was first necessary to observe hatching in order to be certain of their identification. When this was done and the presence of eggs taken into account (with or without lice) the percentage of infestation changed to 76.6 and 79.0 in the 'summer' group and rose as high as 66.7 and 84.2 in the 'winter' group of individuals. It would seem, therefore, that the reproductive cycle has been slowed down with low temperatures and that these lice tend to 'winter-over' in the egg stage as suggested by Peters (1928) for bird lice in general.

Degeeriella nebulosa (ISCHNOCERA: PHILOPTERIDAE): This species remains alive long after the death of its host, and if the bird's skin is kept at room temperature it may be found biting at the plumage even as long as two or three weeks. The *Degeeriella* show some resistance to cold since most survive on bird skins kept in the refrigerator at 10° C. up to the fourth or fifth day. There was no indication of blood in their intestines. These observations support the findings of Wilson (1934) and others, namely, that the feathers alone supply the necessary food and water to the ISCHNOCERA lice. The egg is located on the under surface of the vane of the body feather, usually at a distance of 300–500 μ from the rachis and one to two mil-

TABLE 2.—*Incidence of starlings infected by ectoparasites in eastern North America*

	Date	Source	No. Birds examined	Insecta Mallophaga: *Degeeriella nebulosa* birds infected by adults		*Degeeriella nebulosa* birds infected by eggs		*Menacanthus spinosum* birds infected by adults		*Menacanthus spinosum* birds infected by eggs		Diptera: *Ornithomyia fringillina*	Siphonaptera: *Ceratophyllus gallinae*	Siphonaptera: Parasitoidea	Arachnida Acarina: *Trouessartia rosseri*	Arachnida Acarina: *Rivoltasia* sp.	Arachnida Acarina: *Cheyletiella* sp.
				No.	%	No.	%	No.	%	No.	%	No.	No.	No.	No.	No.	No.
SUMMER	Aug. '45	N. Y.	71	49	69.0	55	77.5	39	54.9	46	64.8	—	—	10	67	—	—
	Sept. '45	N. Y.	64	49	76.6	51	79.7	56	87.5	58	90.6	1	4	1	61	—	—
	Oct. '45	N. Y.	32	16	50.0	22	68.8	25	78.1	29	90.6	—	—	—	32	—	—
WINTER	Nov. '45	Mass.	3	—	—	1	—	3	—	3	—	—	—	—	—	—	—
		Conn.	6	4	44.4	6	77.8	6	100.0	6	100.0	—	—	—	—	2	—
	Dec. '45	N. Y.	1	1	—	1	—	1	—	1	—	—	—	—	—	—	—
		Mass.	18	2	11.5	5	34.6	10	46.2	14	80.7	—	—	—	—	—	—
		Ohio	7	—	—	3	—	1	—	6	—	—	—	—	—	—	—
	Jan. '46	Mass.	11	—	—	—	—	—	—	5	—	—	—	—	—	—	—
		Ohio	7	—	0.0	4	64.3	—	0.0	—	83.3	—	—	—	—	—	—
		Ind.	24	—	—	23	—	—	—	23	—	—	—	—	—	—	1
	Feb. '46	Mass.	3	—	—	1	—	—	—	3	—	—	—	—	—	—	—
		Ohio	5	1	33.3	4	85.2	2	29.6	3	81.5	—	—	—	—	—	1
		Ind.	16	7	—	16	—	6	—	16	—	—	—	—	—	—	10
		Md.	3	1	—	2	—	—	—	—	—	—	—	—	—	—	—
	Mar. '46	N. Y.	8	7	—	7	—	7	—	8	—	—	1	—	6	—	1
	Apr. '46	Mass.	1	—	87.5	—	87.5	—	81.2	—	87.5	—	—	—	—	—	—
		Ind.	7	7	—	7	—	6	—	6	—	—	—	—	7	—	2
	TOTAL		287	144	50.2	208	72.5	162	56.4	234	81.5	1	5	11	173	2	15
	'summer' group		167	114	68.3	128	76.6	120	71.9	133	79.0	1	4	11	160	—	—
	'winter' group		120	30	25.0	80	66.7	42	35.0	101	84.2	—	1	—	13	2	15

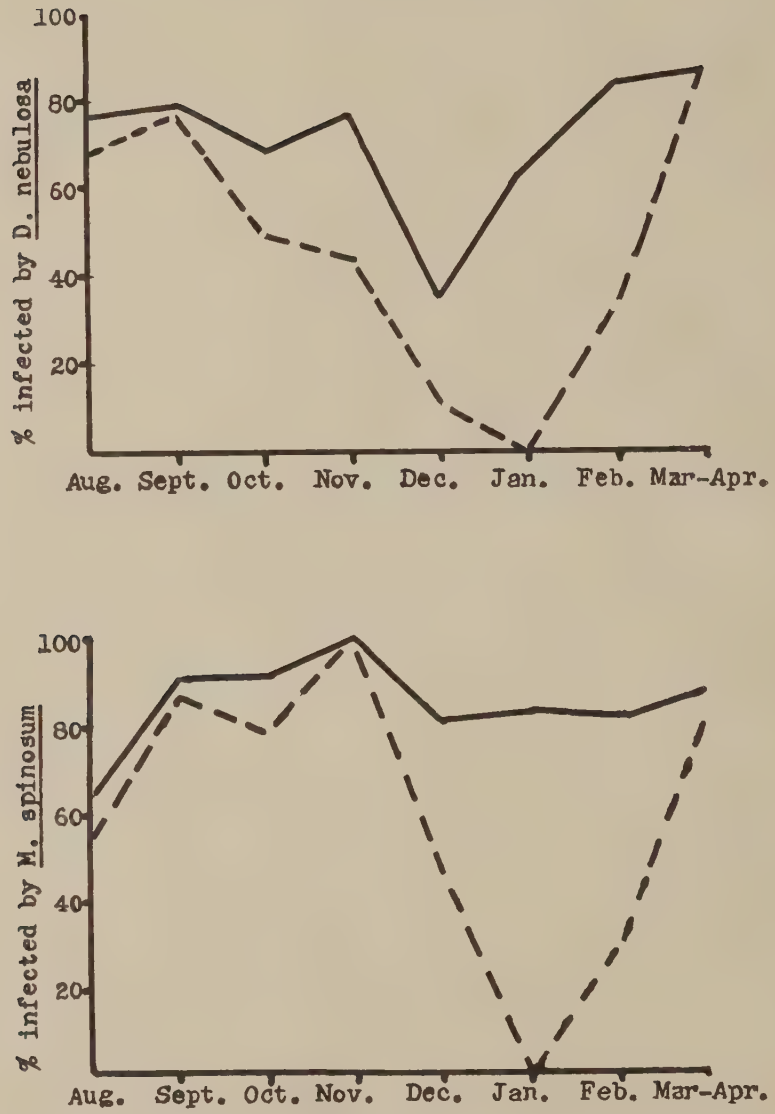


FIG. 1. Percentage incidence of starlings infected by lice, *Degeeriella nebulosa* and *Menacanthus spinosum* between August, 1945 and March–April, 1946.
KEY: ——= presence of eggs with or without lice.
– – –= presence of lice.

limeters anterior to the end of the calamus, so that it is frequently overlapped by the aftershaft (Fig. 2). It is held in place by its base which is encircled and matted-over by the clumping together of barbules from adjacent barbs. In a heavy infestation several eggs may occur on the same feather, arranged one above the other and they may be found on either side of the shaft. The egg is cylindrical in shape, 550 μ

in length with a cap 50–60 μ in height. Scattered irregularly over the cap are 22 micropyles and occasionally a slight thread is evident arising from its peak.

Menacanthus spinosum (AMBLYCERA: MENOPONIDAE): This species is short-lived once its host dies, and is dead by the second or third day even though the bird skin is kept at room temperature or in an incubator at 34° C. This and the fact that the intestines of the nymphs, in particular, were invariably red, substantiate the belief that blood is essential in the diet of the AMBLYCERA lice. The egg is attached by an apparently sticky secretion at its base to the under surface of a body feather at the level of the emergence of the aftershaft, and usually occurs singly or on rare occasions two or three per feather may be present (Fig. 3). It is oval to cylindrical in shape with a cap that terminates in a long tapering thread. The body portion of the egg measures 500–600 μ in length with its greatest width 255 μ. The cap proper is 123 μ high and the slender thread 600 μ long, so that the entire length of the egg is approximately 1.3 mm. The base of the cap is encircled by 14 micropyles, arranged relatively evenly in a single row. The cap on hatching separates from the rest of the egg case directly below the level of the micropyles.

DIPTERA: The fly, *Ornithomyia fringillina* Curtis (HIPPOBOSCIDAE), was taken on only one occasion, from a juvenile in September in New York state (Table 2). This marks the first published record of an hippoboscid fly attacking a starling in North America, though one was reported earlier from the same state in a private communication from Dr. Bequaert. Numerous birds may act as hosts to *O. fringillina,* including the starling in Europe (Thompson, 1937b). Probably the starling with the English sparrow may be responsible for its introduction into this country.

SIPHONAPTERA: The two fleas that have been previously collected from the starling in North America are *Epitedia wenmanni* Rothschild (a flea of the white-footed mouse) and *Ceratophyllus gallinae* Schrank (Fox, 1940). Only the latter was taken by the author (Table 2). Fleas exhibit relatively slight host-specificity compared to the MALLOPHAGA so that the record of *E. wenmanni* on the starling is not too surprising. It has evidently been acquired by the starling since its sojourn here. *Ceratophyllus gallinae* was encountered on four juveniles in September and on a female in April, all from New York state. Only one or two fleas were present per bird. It was observed that they were able to withstand the low temperatures of the refrigerator (10° C.) for at least six days. These constitute the first record in North America of *C. gallinae* taken from the skin of *Sturnus vulgaris* since the one reported by Fox had come from the animal's stomach. This is the commonest flea for the starling in Europe (Thompson, 1937a). According to Ewing and Fox (1943) *C. gallinae* was introduced into North America in the early part of the century, being first discovered in a 'henhouse' at Ottawa in 1909. Possibly the English sparrow or starling played a role in its introduction into this country.

ARACHNIDA

ACARINA: No ticks were found by the author. This is not strange since the majority of the birds came from New York state and those from more southerly states, Maryland, Ohio, and Indiana, numbered only 69 and were collected between December and April. The starling in North America, however, has been found to harbor two species of ticks—*Ixodes brunneus* Koch and *Haemaphysalis leporis-*

palustris Packard (Bishopp and Trembley, 1945). *Ixodes brunneus* is exclusively a bird tick with a large host list. Its original description was based on material from North America. According to Cooley and Kohls (1945, p. 208), "It is not

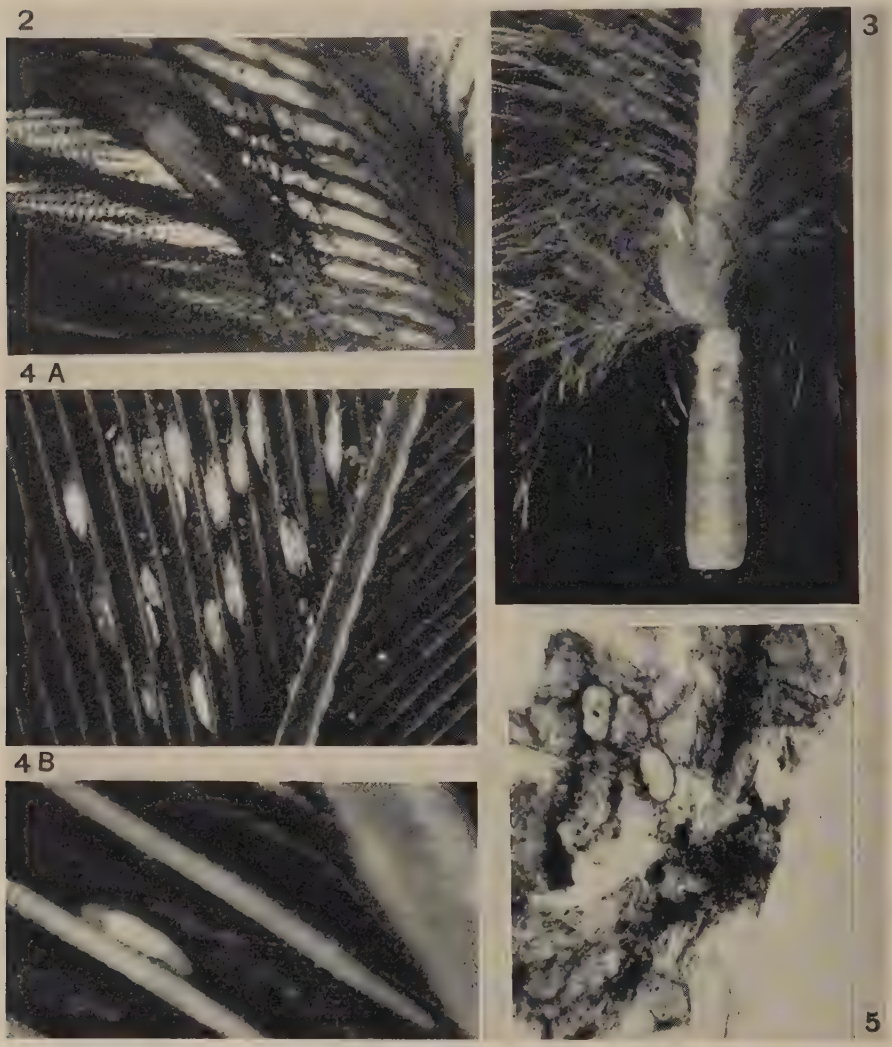


Fig. 2. Egg of *Degeeriella nebulosa* in situ on feather.
Fig. 3. Egg of *Menacanthus spinosum* in situ on feather.
Fig. 4. *Trouessartia rosterii:* A, a cluster in situ on feather; B, egg in situ on feather.
Fig. 5. Egg 'nest' of *Cheyletiella* sp., its protective covering of skin debris having been removed so as to reveal the eggs.

probable that *brunneus* has been carried on birds across the Atlantic Ocean, and it follows that records of this species in Europe and Africa are subject to scrutiny". The rabbit tick of America, *Haemaphysalis leporis-palustris,* is abundant especially in the south and is also widespread among birds. Bishopp and Trembley (1945)

listed 64 species of birds that act as its host, including the starling. Katz (1941) reported it from the starling in Ohio and Peters (1936) for the same bird in Ohio, Virginia and Delaware. It would thus seem that the starling has acquired both these ticks since its sojourn in North America.

Although no ticks were found in the present investigation, mites had infected 67.2% of the birds. For convenience, the mites are grouped into the blood-sucking forms in contrast to the 'feather-mites' that live on the skin and plumage debris and are rarely harmful (Banks, 1915). Two species of blood-sucking mites (PARASITOIDEA) have been reported for the starling in North America—*Atricholaelaps megaventralis* Strandtmann and *Liponyssus sylviarum* C. & F. (LAELAPTIDAE). The former was not observed by the author, but two additional mites, *Dermanyssus gallinae* Deeger and *D. prognephilus* Ewing (DERMANYSSIDAE) were collected. This brings the number of blood-sucking mites to four for the starling in North America (Table 1). Only a few of them were recovered and these were scattered on the plumage of 11 juveniles from New York state in August and September, 1945 (Table 2). The northern fowl mite, *L. sylviarum* is widely distributed throughout the world and has been reported from numerous birds on this continent, including the starling in Canada (Rayner, 1932; Cameron, 1938) and in the United States (Peters, 1936). It was first recorded on this continent in 1920 on poultry and possibly the starling and/or the English sparrow are responsible for its introduction into North America and the same may be true for the chicken mite, *D. gallinae*. The chicken mite is also common and occurs probably wherever poultry are raised (Ewing, 1923). However, the present study marks the first record for the starling in North America. The American species, *D. prognephilus* infests hole-nesting birds such as the eastern bluebird, purple martin, woodpeckers, as well as the cowbird and has been collected from the following states—Maryland, Alabama, Virginia, South Carolina, Michigan, and Ontario (Ewing, 1936). The author reports this species for the first time for the starling and also as a new record for New York state. It has evidently been acquired by this alien bird while usurping the nesting sites of native birds. A similar condition occurs for the mite, *A. megaventralis* which has been taken from hole-nesting animals—squirrels, numerous woodpeckers, from a cliff swallow's nest and in addition from the skins of both the English sparrow and the starling and from 10 states (Ohio and Pennsylvania to Florida and Texas) (Strandtmann, 1947).

Three species of 'feather-mites' were collected by the author, *Trouessartia rosterii* Berl. (EUSTATHIIDAE), *Rivoltasia* sp. (EPIDERMOPTIDAE) and *Cheyletiella* sp. (CHEYLETIDAE) (Table 2), though none has previously been reported for the starling in North America. Since *T. rosterii* is a common parasite of the starling in Europe along with two other species, *Analges passerinus* L. and *Pteronyssus truncatus* Hall var. *quadratus* Berl. (Bonnet and Timon-David, 1934), its presence in North America may be accounted for by its parasitizing those starlings that were imported.

Trouessartia rosterii is ordinarily located on the inner wing surface and lies between adjacent barbs with its head pointing toward the edge of the feather and its posterior end toward the rachis (Fig. 4A). As many as 60% of the starlings harbored this 'feather-mite,' but there was a striking difference in occurrence between the summer and winter months (Table 2). The 'summer' group showed a 96% in-

cidence and the mites were present in great numbers, in particular on the last primaries, the secondaries and on both surfaces of the greater inner coverts. On the other hand, in the 'winter' group only a few were to be found and these occurred on 11% of individuals, which represented the March–April set of birds. They were restricted to the innermost secondary or to the innermost greater covert of the wing. This reduction in prevalence of 'feather-mites' may be the outcome of reduced reproductive activity and development due to the low winter temperatures. The eggs are most frequently to be found on the under surface of the greater coverts within the proximal two thirds of the vane (Fig. 4B). Each egg is attached singly to the distal edge of the barb, thus overlapping the distal barbules of that region, with its long axis parallel to that of the barb. The egg is cylindrical in shape and measures 240 $\mu \times 70$ μ.

Rivoltasia sp. was found on only two birds taken in November, 1945 from Connecticut, and occurred deep down in the ventral body feathers.

Cheyletiella sp. was collected from 15 starlings, in three states—Indiana (13, Jan.–Apr.) ; Ohio (1, Feb.) ; New York (1, March), making an incidence of 5 per cent. The mites were found most frequently on the ventral body plumage. They are difficult to discern with their small yellow bodies and slow crawling movements. They, like the other 'feather-mites,' when few in number on the host, tend to escape detection, so that the percentage of occurrence may be greater than that stated. The eggs of this species of mite are laid singly on the body surface or occasionally in clusters or 'nests' covered over by a few layers of dead stratified squamous epithelium and feather debris. Each 'nest' (Fig. 5) consists of a small elevation of dried, paper-like skin containing within it several eggs and cast skins of mites. The egg is oval, measuring 152–180 μ by 76–83 micra.

ENDOPARASITES

A total of eight endoparasites has so far been recorded in previously published data for the starling in North America (Table 3). This comprises *Isospora* sp., two cestodes (if one discredits two questionable identifications) ; three nematodes and two acanthocephalans. This information has been obtained largely from scattered references in the literature. Two papers, however, deal solely with the starling. One is by Sommer (1936) who retrieved two tapeworms and an acanthocephalan in an examination of the trachea and intestines of 132 birds. The other is a report by Cannon (1939) who took two tapeworms and a nematode from the intestines of 11 individuals. The present investigation adds to the original list an unidentified flagellate, three trematodes, a third cestode, four nematodes and possibly a third acanthocephalan worm (Table 3). A seasonal variation in incidence of tapeworms has been observed and some original and revised descriptions of certain endoparasites have been included.

PROTOZOA

Mastigophora: As far as is known there have been no records for flagellates in starlings. However, an unidentified flagellate was found in enormous numbers amongst the fecal contents of a single adult (Table 4).

Sporozoa: Coccidia of the family Eimeriidae have been recorded for the starling in Europe as *Isospora lacazii* Labbé (1896) and in America as *Isospora* sp.

(Boughton et al, 1938) and, as pointed out by these workers, the genus is cosmopolitan and widely distributed among the PASSERIFORMES. However, its frequency of occurrence in the starling has not been reported. The incidence in the present study is 75% and this high figure is consistent throughout the seasons studied (Table 4). Gross or microscopic lesions caused by the coccidia were not observed. The oöcyst is typically spherical to subspherical in shape with a three layered wall and measures 20.8–28.0 $\mu \times$ 20–27 μ. Sporulation occurred in approximately 24 hours at room temperature. This agrees with observations of Henry (1932) though others (Labbé, 1896; Becker, 1934; Kudo, 1939) stated that it varied from three to, in some cases, 15 days. Each sporocyst is pear-shaped with a prominent knob at the narrow end and contains four sporozoites and a large centrally placed residual body. The aver-

TABLE 3.—*Endoparasites of respiratory-digestive tract for the starling in North America*

Classification Name of parasite	*	x	In present survey		Origin
			% infection	Location in host	
PROTOZOA					
Mastigophora					
Unidentified flagellate	*		0.3	intestine	?
Sporozoa					
Isospora sp.	—		75.3	intestine	Old world
PLATYHELMINTHES					
Trematoda					
Leucochloridium certhiae	*		0.3	cloaca	America ?
Lutztrema sp.	*		0.3	gall bladder	America ?
Brachylaemus sp.	*		0.3	intestine	America ?
Cestoda					
Hymenolepis farciminosa	—		34.0	post. half small intestine	Old world
Choanotaenia musculosa	—		56.3	anterior half	Old world
Paricterotaenia parina	*		5.7	small intestine	Old world
ACANTHOCEPHALA					
Plagiorhynchus formosus		x	4.0	post. half	America ?
Mediorhynchus robustus (*M. grandis*?)		—	2.0	intestine	America
NEMATHELMINTHES					
Capillaria contorta	*	x	11.3	esophagus	Old world
C. ovopunctatum				intestine	Old world
C. exilis	*		60.7	intestine	Old world
Dispharynx nasuta		x	4.3	proventriculus	Old world
Microtetrameres helix (?)	*		0.3	proventriculus	America
Acuaria gracilis v. *sturni*	*		3.3	gizzard	Old world
Porrocaecum ensicaudatum	—		5.7	intestine	Old world
ARTHROPODA-ARACHNIDA					
Acarina					
Speleognathus sturni	—		4.3	respiratory tract	Old world ?

* first published record for the starling in North America.
x parasites also of gallinaceous birds.

age size is 16.6 $\mu \times$ 10.4 μ ranging from 15–18 $\mu \times$ 9.5–12.0 μ. These dimensions and those of the oöcyst fall within the range of measurements for *I. lacazii* (Boughton, 1930; Skidmore, 1934) found in the English sparrow.

PLATYHELMINTHES

TREMATODA: There have been no records of flukes for the starling in North America, except for the experimental infection of a fledgling with *Leucochloridium actitis* following artificial feeding with snails carrying its sporocysts (McIntosh, 1939). In the present study three species of trematodes and an immature form have been found, but each was encountered in only a single bird (Table 4).

Lutztrema sp. (DICROCOELIIDAE). Ten liver flukes were collected from an adult female.

TABLE 4.—*Incidence of starlings infected by endoparasites of the respiratory-digestive tract in eastern North America. First figure signifies total number of birds; figure in parenthesis denotes numbers of juveniles*

Date	Source	Birds No. examined	Protozoa: Mastigophora: Unidentified	Protozoa: Sporozoa: *Isospora* sp.		Platyhelminthes: Trematoda: a. *Lutztrema* sp.	b. *Brachylaemus* sp.	c. *Leucochloridium certhiae*	d. Immature	*Hymenolepis farciminosa*		Whole	
			No.	No.	%	a. No.	b. No.	c. No.	d. No.	No.	%	No.	%
July '44	N. Y.	8(4)	..	6(2)	75	..	..	..	..	2	25	2	25
Dec. '44–Mar. '45	Mass.	5	..	3	60	..	1	..	..	2	40	1	20
Aug. '45	N. Y.	71(66)	..	53(48)	75	..	..	..	..	23(21)	32	23	32
Sept. '45	N. Y.	64(49)	..	48(38)	75	..	..	..	..	22(13)	34	22	34
Oct. '45	N. Y.	32(30)	..	25(24)	78	..	..	..	..	7(7)	22	6	19
Nov. '45	Mass.	3(1)	..	2	44	..	..	..	..	2	33	1	11
	Conn.	6(2)	..	2		1	..	..	..	1		..	
Dec. '45	N. Y.	1	1	..		..	..	..	..	..		..	
	Mass.	18(1)	..	15(1)	81	..	..	..	..	11(1)	54	7	38
	Ohio	7	..	5		..	..	..	..	3		3	
Jan. '46	Mass.	11	..	8		..	..	..	..	1		1	
	Ohio	7	..	5	76	..	..	..	..	1	29	1	9
	Ind.	24	..	10		..	..	..	..	10		2	
Feb. '46	Mass.	3	..	2		..	..	1	..	2		1	
	Ohio	5	..	4	89	..	..	..	..	1	37	..	15
	Ind.	16	..	15		..	..	..	..	5		1	
	Md.	3	..	3		..	..	..	..	2		2	
Mar.–Apr. '46	N. Y.	8	..	4		..	..	..	1	3		2	
	Mass.	1	..	..	62	..	..	..	..	1	44	1	31
	Ind.	7	..	6		..	..	..	..	3		2	
TOTAL		300(153)	1	226(115)	75	1	1	1	1	102(42)	34	78	26
'Summer' group		175	..	132	75	..	..	..	..	54	31	53	30
'Winter' group		125	1	94	73	1	1	1	1	48	38	25	20
PERCENTAGE			0.3		75	0.3	0.3	0.3	0.3		34		26

Description: (Fig. 7). Body semi-transparent, elongate 1.93–2.85 mm. long by 227–386 μ at acetabulum, the maximum width, tapering at the extremities, the anterior narrowing starting at the level of the genital aperture; cuticle smooth. Suckers weakly muscular; oral sucker 87.5–133 μ long by 98–125 μ wide, subterminal to a short lip-like projection of body wall; acetabulum 148–221 μ long by 153–228 μ wide situated within the anterior third of the body. Pharynx shorter than broad, 38–60 μ long by 44–70 μ wide, overlapping the posterior edge of the oral sucker. Esophagus narrow continued posteriorly by a single intestine that takes a zigzag course passing lateral to and between the two testes and between the posterior testis and ovary, keeping dorsal to the vitellaria and uterus and terminating blindly a short distance from the posterior end of the body. Excretory pore terminal. Genital aperture median approximately half-way between the oral and ventral suckers. Cirrus sac elongate, pyriform, 130–188 μ long by 55–86 μ wide, containing a folded seminal vesicle and an eversible cirrus. Testes spherical to transversely oval, never lobed, approximately equal in size, 86–141 μ long by 102–188 μ wide, located diagonally in front of each other in the posterior region of the anterior half of the body. The distances in the different specimens between acetabulum and anterior testis, anterior and posterior testes and posterior testis and ovary show marked variations, namely—94 to 241 μ; 17 to 93 μ, and 7 to 78 μ respectively. Ovary subspherical to transversely oval, 70–92 μ long by 95–123 μ wide, situated off centre about mid-way along the body length. Seminal receptacle small, post-ovarian and median. Vitellaria consist of large follicles that begin just posterior to the ovary and extend posteriorly for a distance of 0.2 to 0.5 mm. Uterus, greatly convoluted, fills practically all of the post-ovarian region and passes forward to the genital aperture keeping in the main to the wavy course of the intestine. Eggs 24–28 μ long by 16–17 μ wide.

TABLE 4.—*Continued.*

Cestoda				Acanthocephala			Nemathelminthes					Arthropoda arachnid	
Choanotaenia musculosa		Whole		*Paricterotaenia parina*	*Plagiorhynchus formosus Mediorhynchus robustus*	*Capillaria contorta*	*Diesharynx nasuta Microtetrameres* sp.	*Acuaria gracilis* v. *sturni*	*Porrocaecum ensicaudatum*	*Capillaria exilis & C. ovopunctatum*		*Speleognathus sturni*	
No.	%	No.	%	No.	No.	No.	No.	No.	No.	No.	%	No.	
6(2)	75	5	62	..	2(2)	..	1(1)	1(1)	2(1)	7(3)	87	..	
2	40	1	20	..	..	..	..	..	..	2	40	..	
43(40)	61	37	52	2(2)	3(3)	7(6)	6(6)	3(3)	9(8)	46(44)	65	..	
38(31)	59	34	53	..	3(2)	8(8)	2(2)	..	2(2)	32(27)	50	3(3)	
19(17)	59	18	56	..	2(2)	5(5)	1(1)	..	..	15(14)	47	..	
3(1)	78	2	22	..	..	..	1	..	..	..	44	..	
4(2)		..		..	..	..	1	..	..	4		..	
1				..	..	..	..	..	..	1		..	
11(1)	58	2	8	..	..	4	..	..	1	4	42	..	
3				..	2	..	1	..	..	6		..	
7				..	..	2	..	..	..	7		..	
1	40	..	0	..	2	2	1	..	..	4	64	1	
9				5	..	..	..	2	..	16		1	
1				..	..	1	..	..	..	1		..	
..	26	..	0	1	1	..	..	..	..	4	18	1	
6				8	..	..	..	1	..	14		1	
..				1	2	..	..	..	..	3		..	
8		7		..	..	5	..	2	3	8		..	
1	94	1	69	..	..	..	..	..	..	1	100	..	
6		3		..	1	..	..	1	..	7		..	
169(94)	56	110	37	17(2)	18(9)	34(19)	14(10)	10(4)	17(11)	182(88)	61	13(3)	
106	61	94	54	2	10	20	10	4	13	100	57	3	
63	50	16	13	15	8	14	4	6	4	82	66	10	
	56		37	6	6	11	5	3	6		61	4	

The only description of a member of this genus so far published in North America is that of *L. monenteron* (Price and McIntosh, 1935) from *Turdus migratorius* and *Sialis sialis* and it differs from the above in certain respects. In the fluke from the starling the testis is entire, not lobed; the egg measures 24–28 μ, not 32 μ; the maximum width of the fluke is 227–386 μ, not 630–670 μ, and the acetabulum occurs within the anterior third instead of being within the anterior fifth of the body as in *L. monenteron.* However, it bears a close resemblance to the liver fluke from the bluejay, *Cyanocitta cristata,* studied by Nesslinger (1950), differing from the latter only in its body length which is 1.93–2.85 mm. in contrast to 4.1–4.7 mm. Possibly it is the same species as that from the bluejay but has not attained full maturity in the starling as host, for the body is foreshortened, and the posterior portion containing the uterine coils is full of eggs, but short in length. A description of the species from the bluejay has been made by Denton and is now in press.[1]

Brachylaemus sp. (BRACHYLAEMIDAE). A single specimen of this intestinal fluke was collected from an adult male.

Description: (Fig. 8). Body cylindrical 2.20 mm long by 0.6 mm wide; cuticle smooth. Oral sucker subterminal, almost spherical, 220 μ long by 195 μ wide. Acetabulum smaller than

[1] Private communication from Dr. McIntosh.

oral sucker, 183 μ long by 190 μ wide, located so as to divide the body 1 : 3. Pharynx subspherical, 108 μ long by 132 μ wide, overlapping the posterior edge of the oral sucker. Esophagus very short, intestinal ceca extend to within 70 μ of the posterior end. Genital aperture median, 55 μ anterior to anterior testis. Gonads median, tandem and overlapping each other in the posterior third of the body. Anterior testis 241 μ long by 250 μ wide; posterior testis 269 μ long by 228 μ wide. Ovary between the testes—spherical 188 μ diameter. Oviduct originates from the median posterior portion of ovary. Vitellaria extracaecal, starting at level of 160 μ posterior to the anterior edge of the anterior testis and extending forward to 35 μ beyond the acetabulum. Eggs 33–38 μ long by 14–20 μ wide. This is probably a young form as no eggs are in close proximity to the genital aperture.

The intestinal fluke, *B.* (*Harmostomum*) *fuscatus* that occurs in the starling in Europe (Baylis, 1928) differs from the above species in the following points: 1) the vitellaria terminate posterior to the acetabulum; 2) the anterior testis overlaps the genital aperture; 3) the gonads are well removed from each other; and 4) the egg measures 22.8 μ × 11.4 μ (Braun, 1902). The fluke here described bears closest resemblance to *B. mcintoshi* Harkema (1939) from the barred owl, but in the latter the body is narrower (0.29 mm.), the suckers and pharynx are smaller (210 μ × 163 μ; 130 μ × 121 μ; 77 μ × 95 μ, respectively), the pharynx does not overlap the oral sucker and the testes are contiguous. This last difference, however, may be accounted for by the fact that the intestinal fluke in the starling is probably a young specimen.

Leucochloridium certhiae McIntosh (Brachylaemidae): The cloacal fluke was retrieved from an adult female. This fluke was first described in 1927 from the brown creeper and formed the first record of the genus in North America (McIntosh, 1927); later McIntosh (1932) pointed out that it is identical or closely related to one described as *Leucochloridium* sp. from the spotted flycatcher, *Musciapa striata* (Witenberg, 1925) in Europe. Species of *Leucochloridium* to date have not been encountered in the starling in Europe, although it infects a variety of Passeriformes. Might it be the same species and might the starling have played a role in its introduction into North America?

Two immature flukes were found in the intestine of an adult female in April, 1946. Judging from the body shape, the strong large suckers, prominent pharynx, character of the intestinal crura and position of the gonad primordia, along with the fact that they were found in the intestinal tract, it is possible that they are immature forms belonging to the genus *Leucochloridium.*

Cestoda: The first of the two tapeworms taken from starlings in North America was *Hymenolepis farciminosa* Goeze by Chapin (1920) and later by Sommer (1936) and Cannon (1939). The latter worker at the same time collected the other cestode, *Choanotaenia musculosa* Fuhrmann. Sommer called the second tapeworm found in his study *Rhabdometra nullicollis.* Such identification should be questioned since this species is a parasite of grouse (Galliformes). The same holds true for the report of *Monopylidium* sp. for the starling by Rayner (1932). Considering the fact that maceration of the scolex of *C. musculosa* readily occurs and its proglottids somewhat resemble those of *Rhabdometra,* the writer believes that the tapeworm found by Rayner and the one identified as *R. nullicollis* by Sommer is *C. musculosa.* In Europe *C. musculosa, H. farciminosa* and *Aploparaksis dujardinii* Krabbe constitute the tapeworms of *Sturnus vulgaris.* Occasionally four more, that typically parasitize other members of the Passeriformes, may infect this host, of which one is *Paricterotaenia parina* Duj. of the English sparrow (Baylis, 1928, 1939; Joyeux and Baer, 1936; Sprehn, 1932).

In the present investigation, three species of tapeworms were collected,—*H. farciminosa* (Hymenolepidae) and two members of the family Dilepidae, *C. musculosa* and *P. parina* (Table 4). The finding of *P. parina* forms the first record of its occurrence in the starling in North America. Its failure to reach maturity in this host and its low incidence (6%) would indicate that the starling is probably not responsible for its presence in this country, but that the responsibility lies with its natural host, the English sparrow. Undoubtedly the introduction of *C. musculosa* and *H. farciminosa* is due to *Sturnus vulgaris*. The incidence of birds infected by tapeworms was high, 71%. Sommer (1936) obtained a much lower figure, 39% with 1.14 as the average number of specimens per bird, the maximum being 15 in any one bird. The general health of the starling seemed unimpaired by the presence of even a heavy infection by cestodes. The adult that harbored 101 *C. musculosa* and 15 *H. farciminosa* appeared in good condition and the juvenile with 125 *C. musculosa* and two *H. farciminosa* was extremely plump. Microscopically it is evident that the tapeworm can penetrate as far as the glands of Lieberkühn, destroying the mucosa in its path (Fig. 15).

Hymenolepis farciminosa: The size of its 10 hooks as given by Krabbe (1869) is 20–23 μ; the author found them to be 23–25 μ (Fig. 9A). The embryo has been said to measure 80 $\mu \times 60$ μ (Joyeux and Baer, 1936; Hughes, 1941). However, this figure was given for the egg in its original description (Volz, 1900) and has been confirmed by the writer, namely—egg, 76–88 $\mu \times 69$–70 μ; embryo, 48–54 $\mu \times$ 40–45 μ; its hooks 18–20 μ. This long delicate worm lies coiled on itself in the posterior half of the small intestine. The maximum number of worms per bird was seven with an average of two, but when scolices only were present the number may be much higher—79 on one occasion. The percentage of infected birds was 31 and 38 for the 'summer' and 'winter' groups, respectively. However, when the presence of whole worms only was counted the figure was 30% and 20%, respectively (Fig. 6; Table 4). Thus a seasonal difference occurred though it was less striking than for *C. musculosa.*

Choanotaenia musculosa: There is disagreement as to the character of the hooks and egg of *C. musculosa* and neither has been illustrated. In the original account, Fuhrmann (1896) was unable to describe the nature of the hooks due to ready maceration of the scolex and gave 180 μ as the diameter of the egg (possibly this represented its uterine capsule). Joyeux and Baer (1936) stated that the hooks number 22, occur in two rows and measure 28 μ and 24 μ; and that the egg measures 50 μ, the embryo 30 μ and its hooks 18 μ in length. Cannon (1939) failed to obtain a complete crown of hooks, but noted that they were in two rows and measured 43 μ; he included no account of the egg. The writer was fortunate in securing ample fresh material and as a result is now able to redescribe and illustrate these portions. The hooks (Fig. 9C) number 18–20 and are arranged in two rows; those of the proximal row measure 36.6–43.5 μ and of the distal row, 43–48 μ. The egg (Fig. 10) has a diameter of 47–55 μ, the enclosed embryo 32–36 μ and its hooks are 13–14 μ in length. *Choanotenia* is localized in the anterior region of the small intestine. The percentage of infected birds was 56, but a seasonal difference was apparent both in the numbers of infected birds and in the presence of complete worms. The 'summer' group exhibited a 61% infection, and 54% possessed whole worms. In contrast to this, the 'winter' group showed a 50% incidence (43% omitting March–April) and only 13% (or

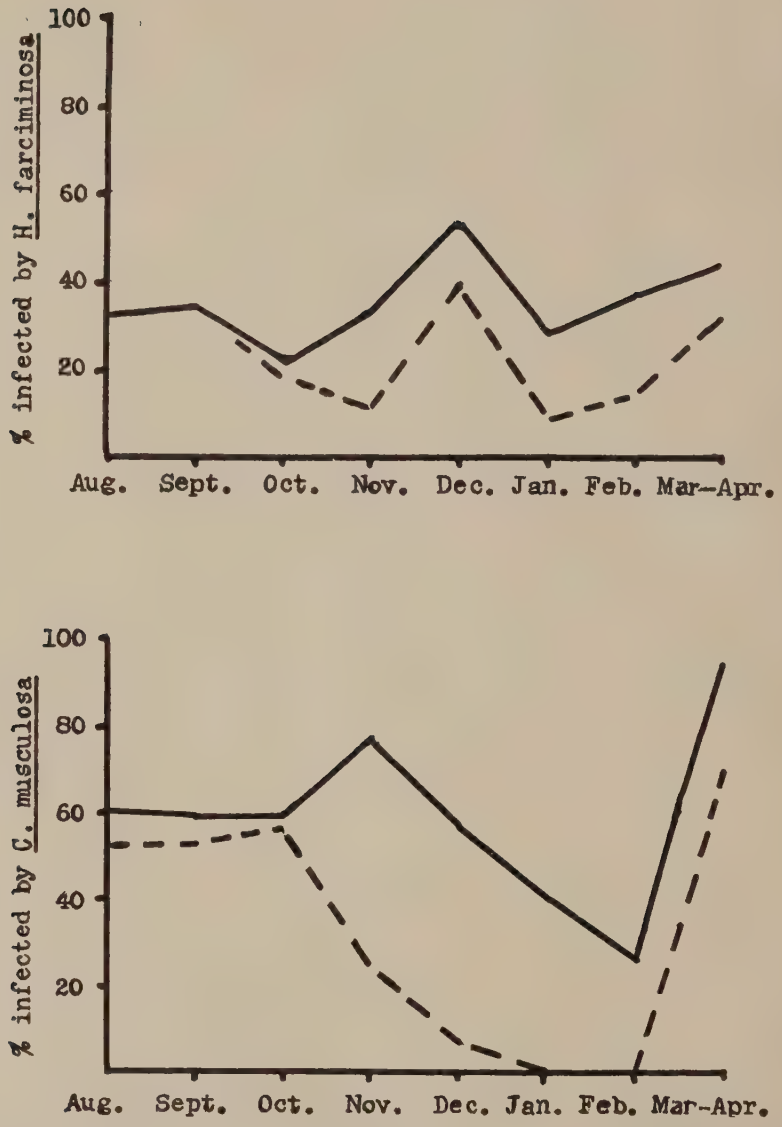


FIG. 6. Percentage incidence of starlings infected by tapeworms, *Hymenolepis farciminosa* and *Choanotaenia musculosa* between August, 1945 and March–April, 1946.
KEYS ——= presence of worms, both fragments and whole.
– – –= presence of whole worms only.

5% omitting March–April) harbored whole worms (Fig. 6; Table 4). The infestation in individual birds was heavy, 13 being the average per bird, though as many as 125 occurred in one instance.

Paricterotaenia parina: The identification of this species of tapeworm is based purely on the nature of the scolex and maturing proglottid, since none had reached sexual maturity. The shape and size (18–23 μ) of the hooks (Fig. 9B) agree with

those described by Krabbe (1869) who pointed out that the size varies with its host, being 15–17 μ in *Parus coeruleus* and 21 μ in *Sturnus vulgaris*. The tapeworms were located in the anterior region of the small intestine and often occurred merely as scolices, ranging from one to eleven, although in three cases the number reached 36, 39 and 164 per bird. They occurred in only 6% of individuals but these were widely distributed (N. Y., Ohio, Md., Ind.) (Table 4).

ACANTHOCEPHALA

Up to the time the present investigation was undertaken, two species of ACANTHOCEPHALA had been taken from *Sturnus vulgaris* in North America. Sommer (1936) collected three worms from 132 individuals examined from Illinois and identified them as *Mediorhynchus grandis* Van Cleave. Van Cleave (1942) found *Plagiorhynchus formosus* Van Cleave in a single bird from New Jersey. ACANTHOCEPHALA exhibit relatively slight host-specificity, so that a species may be found in a variety of different birds. In Europe *Prosthorhynchus transversus* Rud. (Baylis, 1928) and *Mediorhynchus micracanthus* Rud. (Sprehn, 1932) have been reported as parasites of the starling.

The writer obtained a 6% infection of starlings by ACANTHOCEPHALA and both genera were represented. None of the birds involved came from Massachusetts or Connecticut. Seven was the maximum number present at any one time and 2 the average number per bird. The starlings appeared to suffer from such infections for their bodies tended to be emaciated and the visceral contents black. The worms surpassed all others in their ability to penetrate the host's tissue for they were able to extend deep into the muscular coat and to lie in close proximity to the serosa (Fig. 16). Their point of attachment was at times visible on the external surface of the intestine.

Plagiorhynchus (*Prosthorhynchus*) *formosus* (RHADINORHYNCHIDAE): This acanthocephalan was found in 12 birds representing five each from New York and Ohio and two from Maryland. The Ohio findings constitute a new state record for this parasite. Van Cleave (1942) pointed out that with its restricted distribution and the fact that its intermediate host, *Armadillidium vulgare* is widely distributed throughout America, possibly *P. formosus* is not an American form, but has been introduced with its definitive host into this country. Could the definitive host be *Sturnus vulgaris?*

Mediorhynchus robustus: This worm was found on six occasions, five times in birds taken in New York and once in a bird from Indiana. These data, host and state records, with the addition of New Jersey, were published for the first time in a paper on *Mediorhynchus* by Van Cleave (1947). In the article (Van Cleave, 1947), the starling was omitted in the host list for *M. grandis* although Sommer (1936) reported its presence in this host in Illinois. Possibly Sommer's *M. grandis* should have been identified as *M. robustus* or the starling may serve as host to both these species of *Mediorhynchus*.

NEMATHELMINTHES

Three nematodes so far have been reported for *Sturnus vulgaris* in North America,—from the proventriculus, *Dispharynx nasuta* Rud. (Goble and Kutz, 1945a); and from the intestine, *Porrocaecum ensicaudatum* Zeder (Cram, 1933) and

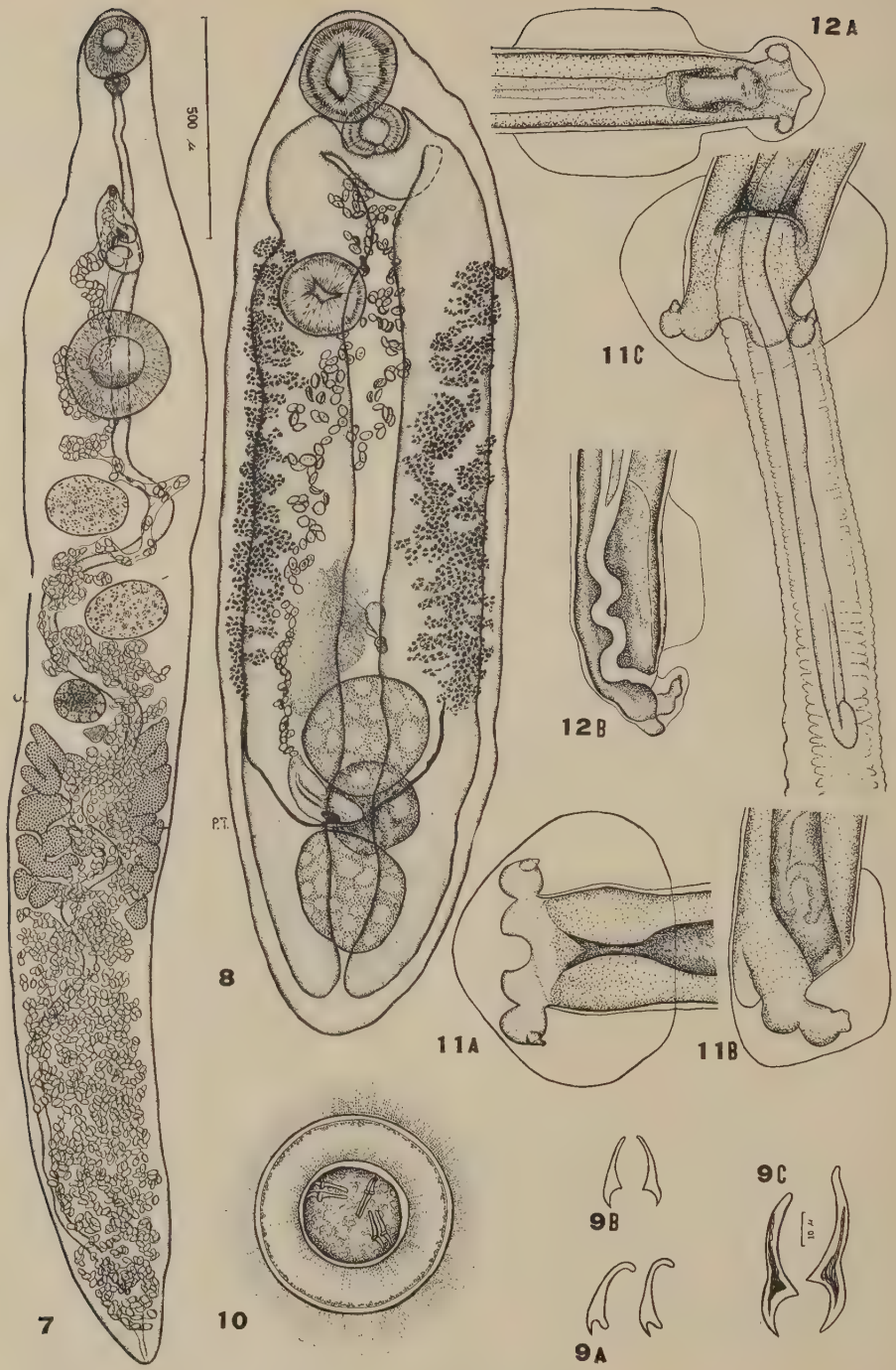


FIG. 7. *Lutztrema* sp., ventral.

FIG. 8. *Brachylaemus* sp., ventral.

FIG. 9. Hooks of tapeworms: A, *Hymenolepis farciminosa;* B, *Paricterotaenia parina;* C, *Choanotaenia musculosa.*

FIG. 10. Egg of *Choanotaenia musculosa,*—fresh preparation.

FIG. 11. *Capillaria ovopunctatum,* cauda of male: A, spicule sheath retracted, ventral; B, spicule sheath retracted, lateral; C, spicule sheath everted, ventral.

FIG. 12. *Capillaria exilis,* cauda of male; spicule sheath retracted: A, ventral; B, lateral.

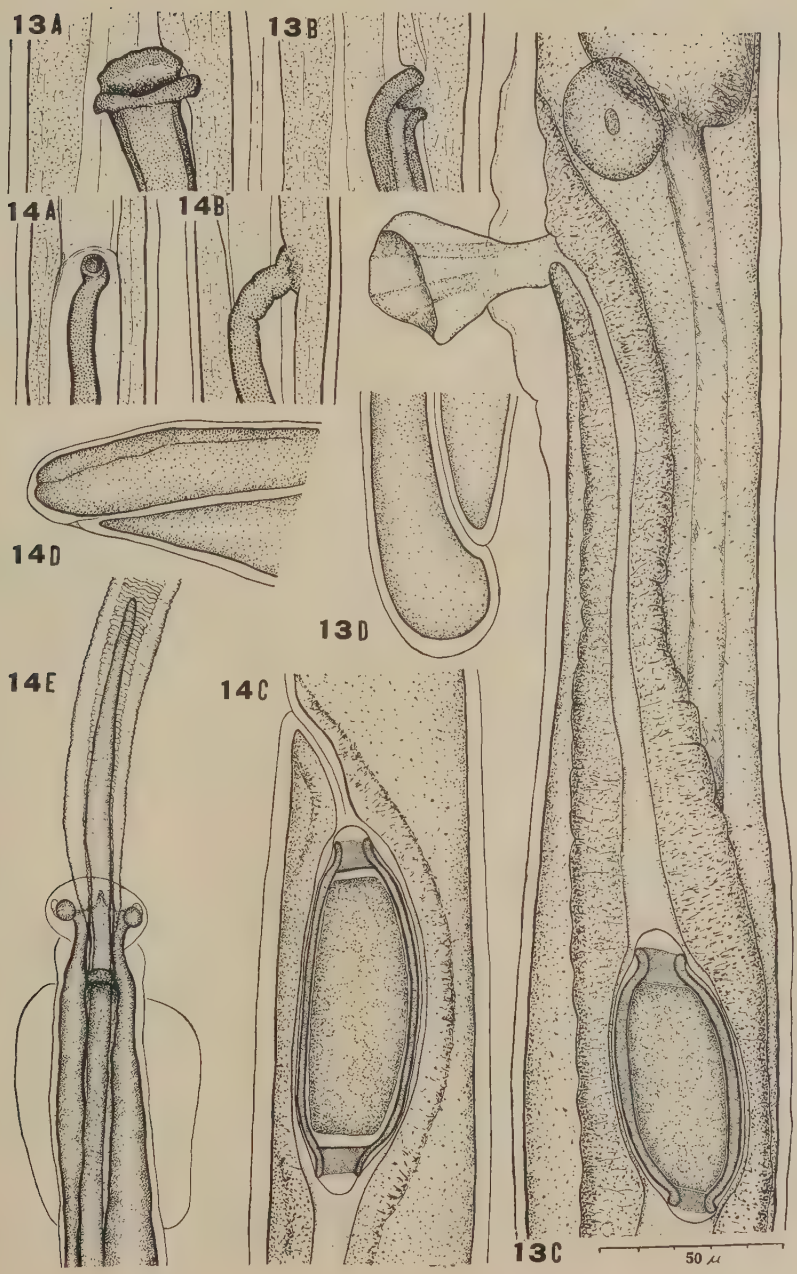
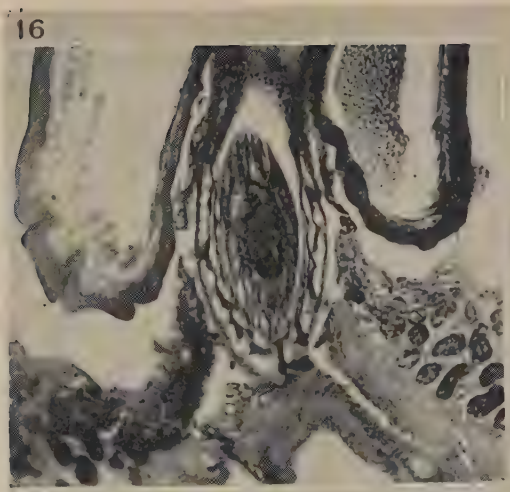
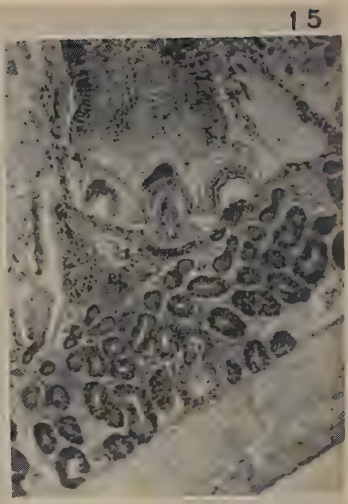
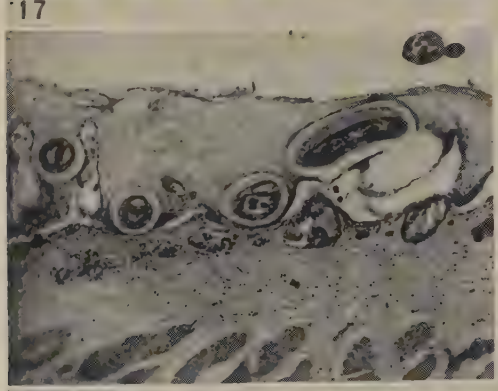


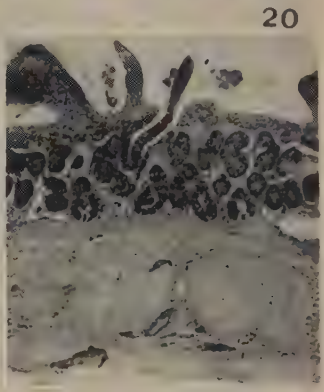
FIG. 13. *Capillaria ovopunctatum:* A, male, proximal end of spicule, ventral; B, male, proximal end of spicule, lateral; C, female, vulvar region, lateral; D, cauda of female.

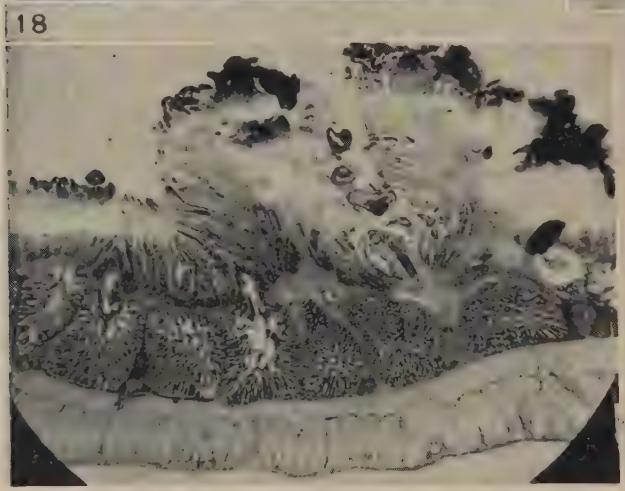
FIG. 14. *Capillaria exilis:* A, male, proximal end of spicule, ventral; B, male proximal end of spicule, lateral; C, female, vulvar region; D, cauda of female; E, male, spicule sheath everted.











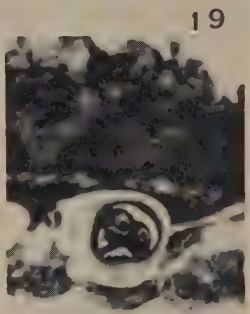


FIG. 15. Section small intestine passing through area of attachment of *Choanotaenia musculosa.*

FIG. 16. Section of small intestine at point of insertion of *Plagiorhynchus formosus.*

FIG. 17. Section of esophagus containing *Capillaria contorta.*

FIG. 18. Section of proventriculus showing marked pathological condition of the mucosa due to the presence of *Dispharynx nasuta.*

FIG. 19. Section of inner tunica of gizzard parasitized by *Acuaria gracilis* var. *sturni.*

FIG. 20. Section of small intestine through region of insertion of an intestinal *Capillaria.*

a species of *Capillaria* (Cannon, 1939; Read, 1949). The latter was tentatively named *C. columbae* var. *sturni* by Cannon and identified as *C. caudinflata* Molin by Read. These worms have been collected by the author but the *Capillaria* has been recognized as representing the two species *C. ovopunctatum* v. Linstow and *C. exilis* Duj., which, with the exception of *D. nasuta* and the addition of *Syngamus trachea* Montague, constitute the roundworms for the starling in Britain (Baylis, 1928). The author also found the esophageal *Capillaria* (*C. contorta* Creplin); the gizzard worm (*Acuaria gracilis* Gendre) and immature forms in the stomach and the intestine (Table 3). This makes a total of at least six roundworms for this host in North America. The nematode list for the starling in the rest of Europe is similar to that compiled by Baylis for Britain with the inclusion of *C. contorta, Viguiera turdi* Molin (Gizzard worm) and another *Porrocaecum, P. heteroura* Creplin (Sprehn, 1932). *Dispharynx nasuta* has been reported by Osersky (1927) from the starling in Asia along with two gizzard worms, *A. anthuris* Rud., and *A. sturni* Osersky.

In the present study as many as 68% of the hosts harbored nematodes, although the incidence of such infections recorded in the literature is meagre for this and other wild birds. This high figure may be accounted for by the fact that fresh material was always examined and all but one of the roundworms are slender and would be extremely hard to discern in the preserved condition. Except in two instances, tissue damage resulting from nematode infections appeared to be negligible. Gapeworms, *S. trachea,* were entirely lacking in the present study. Goble and Kutz (1945b) also found this to be true on dissecting 118 starlings. The absence of *Syngamus* in this host in America is surprising since in Britain Lewis (1925, 1926) obtained a 35% infection following an examination of 520 starlings, a much higher occurrence than in any other wild bird. The worms in the following account have been discussed in a definite sequence, namely those parasitizing the esophagus, proventriculus, gizzard and finally the intestines.

Capillaria contorta (Trichuridae): This nematode was found in the esophagus of 11% of the starlings. No seasonal difference in occurrence was apparent but it was noted that they were absent in hosts from Indiana, Connecticut and Maryland (56 birds in all) whereas the incidence in birds from Ohio, New York and Massachusetts ranged from 10% to 17% (representing 244 individuals) (Table 4). Infections were light as the number of parasites present at any one time never exceeded seven, and averaged 1.7 per infected bird. The worm was located within the anterior half of the esophagus between adjacent longitudinal folds of the wall. The sinuous contour that each assumed would allow for the contraction and expansion of this region during food intake. Unlike the majority of helminthic infections, *C. contorta* typically was found imbedded in the mucosa throughout most of its body length. Microscopic preparations revealed that the worm failed to penetrate beyond the stratified squamous epithelium (Fig. 17). In some cases, the inner surface of the esophagus exhibited vascular patches and a catarrhal condition. Microscopically the infected area showed a thickening and often a sloughing of the mucosa accompanied by an infiltration of leucocytes. The above data constitute the first record for *C. contorta* for the starling in America. This worm is world-wide in distribution parasitizing numerous birds including gallinaceous and passerine forms. It was first found in North America in 1929 in the quail (Cram, 1936) and as none has

been found here in the English sparrow (Hopkins and Wheaton, 1935) the starling may have been responsible for its occurrence in America.

Dispharynx nasuta (Acuariidae): This species of *Dispharynx* is widely distributed throughout the world and is known to parasitize the proventriculus of members of the Galliformes, Columbiformes and Passeriformes. Its first appearance in the United States was in 1913 and Goble and Kutz (1945a) included the starling in the list of 20 avian hosts for this worm in North America. They obtained a 5% infection in the starling as a result of an examination of 118 individuals collected between March and December 1944. In the present study 13 starlings were found to harbor *Dispharynx* but there appeared to be a slight seasonal variation in its occurrence. In the 'summer' group there was a 5% incidence (an average of 2 per infected bird) but a 3% for the 'winter' group and never more than one worm per bird. Two hosts revealed a marked pathological condition. The region of attachment of the worm had undergone proliferation so that it projected 1 mm into and partially obliterated the lumen (Fig. 18). Liquefaction and necrosis had set in with infiltration of leucocytes and excessive mucous secretion. A similar picture from such infections has been described in other hosts in particular in the grouse (Allen, 1924). The degree of proventriculitis is said to be correlated with the number of worms present (Goble and Kutz, 1945a). However, only a single worm was found in each of the two cases described above, although up to seven were present in other starlings without any apparent impairment. One of the 13 infected hosts collected in January from Ohio was parasitized by what is taken to be the third larval stage of *D. nasuta,* since it bears a close resemblance to the description as given by Cram (1931) for the infective stage of this species. Its round head lacks all evidence of cordons but possesses two small pointed lips with four papillae; its body measures 3.2 mm with a pharynx of 100 μ and an esophagus of 750 μ, and it is in the process of molting.

Microtetrameres helix (Tetrameridae): An additional starling, a juvenile caught in August, was found to contain 10 larvae in its proventriculus differing markedly from *D. nasuta.* Their tails terminate in a characteristic ball-shaped knob and the body measurements are as follows: body length, 2.14–2.8 mm; tail, 250–285 μ; buccal cavity, 20–24 μ; muscular part of esophagus, 170–219 μ; glandular part of esophagus, 438–530 μ. They have been tentatively identified as larvae of the crow stomach worm, *M. helix* (?) as this description closely parallels that for the infective larva of this species (Cram, 1934b).

Acuaria gracilis var. *sturni* (Acuariidae): Gizzard worms were encountered in 10 starlings though hitherto none has been reported for this bird in North America (Table 4). Their coiled bodies lay imbedded between the 'horny' layer and the mucous membrane of the inner tunica (Fig. 19), and on their removal would typically leave an undulating impression on this and occasionally also on the outer tunica. This coiling of their bodies is reminiscent of *Capillaria contorta* and undoubtedly aids in conforming to the muscular movements of the gizzard. Their presence appeared to cause little or no pathological condition. The worms were mature in eight of the hosts and have been identified as *Acuaria gracilis* var. *sturni.* They are identical with *A. gracilis* from the African birds, *Buchanga atra* and *Oriolis auratus* (Cram, 1927), except that the cordons in the male are 1.9–2.07 mm, that is 1/3 of

the body length, and the tail 1/15–1/20 of the body length (220 μ and 1/40, respectively, in *A. gracilis*). Great variation, in cordon length however may occur within a single species (Shikhobalova, 1930). Unidentified larval forms occurred in two birds killed in July 1944 and April 1946. The larva in the July host possesses four cordons directed posteriorly for a distance of 66.6 μ; its esophagus is 333 μ in length and its body 2.32 mm long. The bodies of the two specimens from the April bird measure 4.5 and 4.9 mm, and the tails 153 and 156 μ respectively. The esophagi are 582 and 585 μ in length and the head of each possesses two lips and a cuticular collar. This latter feature is present in the third larval stage of *A. anthuris* (Cram, 1934a), but the spiny character of the tail present in this species is lacking in the specimens from the starling.

Porrocaecum ensicaudatum (Ascaridae): The intestinal roundworm, *P. ensicaudatum* has been reported for the starling in Canada (Cram, 1933) but not in the United States although it is prevalent in numerous birds here and in other parts of the world including the starling in Europe. In the present investigation it occurred in 17 hosts from Massachusetts and New York, 11 of which were juveniles (Table 4). Infection was light, the average number per bird being 2, and five the greatest number in any one bird. Immature forms, probably of this species, parasitized four birds killed in August 1945 and April 1946. They possess a body 3.76 mm in length; an anus, 220 μ anterior to tail extremity; a buccal cavity, 173 μ long and an esophagus, 85.3 μ in length.

Capillaria ovopunctatum and *C. exilis* (Trichuridae): Two intestinal threadworms have been listed for the starling in Europe,—*C. ovopunctatum* described by von Linstow (1873) and *C. exilis* by Dujardin (1845). Although there exists no modern description, the meagre original descriptions are adequate to distinguish them from each other. The males differ in the size of the body and the spicule and in the presence or absence of caudal alae; whereas the females differ in the size of the egg and in the presence or absence of the vulvar appendage (Table 5). In this country intestinal *Capillaria* have been reported for this host by Cannon (1939) and Read (1949). Cannon collected several specimens from 11 starlings in Quebec and tentatively named them *C. columbae* var. *sturni* since they closely resembled *C. columbae* Rud. except in the presence of the vulvar appendage, which is absent in the threadworm of the pigeon. Read found two females in one of 25 starlings from Michigan and identified them as *C. caudinflata* Molin as they conformed to the description of this species. He also suggested that Cannon's specimens be referred tentatively to *C. caudinflata* due to the possession of the vulvar appendage.

In the present investigation, as many as 182 birds were infected by intestional *Capillaria*. It was obvious on microscopic observation that they represented two distinct species and are believed by the writer to be none other than the European forms, *C. ovopunctatum* and *C. exilis*. For comparative purposes a table has been compiled to demonstrate the salient features of the *Capillaria* under discussion (Table 5). The ratio of the length of the esophagus to the body length or to the intestinal length has been omitted as a specific character since this varies with the age of the individual (Morgan, 1932). It is evident (Table 5) that the two species collected by the author more closely resemble the European forms than either *C. caudinflata* or *C. columbae,* and they closely parallel the original descriptions of *C. ovopunctatum* and *C. exilis* as given respectively by von Linstow and Dujardin.

Cannon's and Read's specimens may prove to be *C. ovopunctatum* and it was thought wiser to omit them in Table 3. Read's identification was based solely on females, as males unfortunately were not available to him. A more detailed account with illustrations is now presented for these intestinal *Capillaria* of the starling.

Capillaria ovopunctatum von Linstow (Figs. 11A-C, 13A-D; Table 5).

Male: Caudal alae absent. Spicule 0.8–0.95 mm. in length, proximal end expanded laterally with a central groove, distal end terminated in a recurved point. Bursa expanded anterolaterally and supported by a pair of rays each of which carries a secondary outgrowth. Tail with a median terminal depression.

Female: Vulvar appendage present, varies in shape, but most frequently funnel-shaped with a width of 38–55 μ. Vulva usually 15–25 μ posterior to esophagus. Egg 59–65 μ long by 24–29 μ wide.

TABLE 5.—*Comparison of* Capillaria caudinflata, C. columbae *and intestinal* Capillaria *from the starling*

	Male body length in mm.	presence of caudal alae	spicule length in mm.	shape bursal rays	Female body length in mm.	presence of vulvar appendage	Egg	
							length in μ	breadth in μ
C. caudinflata	9.0–25.0	+	0.8–1.1	T-shape	14.0–40.0	+	53	23
C. columbae	8.4–13.8	–	1.0–1.7	L-shape	10.0–17.2	–	44–62	20–27
C. columbae var. *sturni* (Cannon)	7.0–17.25	–	approx. 1.0	secy. out-growth	9.5–18.25	+	48–52	22–23
C. ovopunctatum (von Linstow)	6.24	–	0.9	2-lobed	9.5	+imm.	59	29
C. ovopunctatum (Boyd)	7.0–10.0	–	0.8–0.95	secy. out-growth	10.0–12.6	+	59–65	24–27
C. exilis (Dujardin)	9.5	+	1.0	?	9.6	–	72	34.5
C. exilis (Boyd)	9.9–12.0	+	1.2–1.3	lobed	11.4–11.9	–	71–78	33–35

Capillaria exilis Dujardin (Figs. 12A, B; 14A–E; Table 5).

Male: Caudal alae present, 37–39 μ long by 17–20 μ broad, emerging 8–15 μ anterior to bursa. Spicule 1.0–1.3 mm. long, not expanded proximally, ending in a straight point distally. Bursa relatively small, supported by a lobed ray on each side. Tail with a small median terminal outgrowth.

Female: Vulvar appendage absent. Vulva at level of junction of esophagus with intestine. Egg 71–78 μ by 33–35 μ.

Numbers of the two species collected in individual birds were not recorded but *C. ovopunctatum* appeared to be far more prevalent than *C. exilis*. There were fewer males present than females (349 : 403). Graybill (1924) also noted that females predominated in his work on *C. columbae* from the chicken and turkey. The percentage incidence was slightly higher in adult hosts than juveniles (64 : 57) and accounted for the lower percentage of infection, 57, of the 'summer' group of starlings and the higher percentage, 66, in the 'winter' individuals. Infestation was heavier in birds collected from certain states than from others: 79% of birds from Indiana were infected; 59% from New York but only 37% from Massachusetts. The average number per bird was 4 though on one occasion as many as 68 worms were encountered. The intestinal *Capillaria* is able to penetrate deep into the glands of Lieberkühn and by so doing causes a negligible degree of destruction (Fig. 20). The host's tissue responds by the development of a thin connective tissue layer encircling

the implanted portion of the parasite and by a slight infiltration of leucocytes into the area.

Evidently *C. ovopunctatum* and *C. exilis* have been brought into North America along with the starling. Since both species have spread to members of the TURDIDAE in Europe and in the case of *C. exilis* to the pheasant also (Baylis, 1939), it will be interesting to note whether in time they will be observed in native birds in this country. Other nematodes that may have been introduced into America by this bird are *C. contorta, Dispharynx nasuta, Acuaria gracilis* and *Porrocaecum ensicaudatum.*

ARACHNIDA—*ACARINA*

Speleognathus sturni Boyd: A new species of mite was encountered in the trachea of 13 starlings. This mite has since been described as *S. sturni* (Boyd, 1948). Further dissection of the hosts revealed their presence in large numbers in the nasal cavities. Mites identical to this species were collected by C. D. Radford[2] in 1945 from the Mynah birds (*Manipur imphal* and others) in India while working with the scrub-fever commission. These birds belong to the same family as does the starling, namely the STURNIDAE, which has its major locale in the Indian and Ethiopian regions. Possibly the mite gained access to North America through the starling and has now spread to native birds such as the boat-tailed grackle, *Cassidix mexicanus* (Boyd, 1948).

DISCUSSION

The incidence of parasitism ranks high in *Sturnus vulgaris* particularly in the spring to fall months, and from this aspect it would be good source material for a course in parasitology. The prevalence of parasites is dependent on numerous factors, such as diet, habits, age and habitat of the respective host. The fact that the starling is omnivorous in its diet is in itself one of the main causes for its numerous parasites, both in quantity and diversity of species. The extremely low incidence of trematode infection in this host in America, possibly much lower than for the same bird in Europe, can probably be accounted for by the fact that less than one per cent of their animal food consumption consists of snails whereas in England the bird devours large quantities of molluscs (Kalmbach and Gabrielson, 1921). The habit of the starling of feeding and roosting with native birds possibly has been the cause of its acquiring the louse, *Degeeriella illustris* from grackles and the transference of its louse, *D. nebulosa,* to the Eastern robin. Similarly, its objectionable habit of usurping nesting sites of native animals may account for its acquisition of certain of their blood-sucking ectoparasites, namely, the flea, *Epitedia wenmanni,* the ticks, *Haemaphysalis leporis-palustris* and *Ixodes brunneus* and the mites, *Dermanyssus prognephilus* and *Atricholaelaps megaventralis* (Table 1). The age of the host is often important in the prevalence of a parasite for the host may acquire an immunity which would control the longevity of the parasite. Although 175 out of the 300 starlings were immature the endoparasitic picture was very similar to that in adults except in the case of the intestinal *Capillaria* and *Porrocaecum ensicaudatum,* where 57 and 7% juveniles were infected to 64 and 4% adults.

The habitat of the host, in particular the climate of its environment, may affect the presence of parasites, either directly by its influence on the ectoparasite and the

[2] Personal communication.

free-living stage of the endoparasite or indirectly by its control of their intermediate hosts. The relation of meteorological factors to parasitic incidence in starlings was demonstrated by Markov (1940). His study consisted of an examination of 215 birds including nestlings between April and October 1935 and 1936 at Leningrad where starlings are migratory, being absent in winter. He noted that the dry weather of June 1936 acted as a climatic barrier by destroying the spores and eggs of *Isospora* and *C. contorta* respectively. However, he stated that the ectoparasitic fauna fluctuated with the biology of the bird, the majority of the parasites being lost during the molting process of the host in the fall, and that ectoparasites are not affected by climatic conditions. The present findings disagree with the latter statement for a definite seasonal fluctuation was apparent for both species of MALLOPHAGA irrespective of molting (Fig. 1) and the same holds true for the 'feather-mite' *Trouessartia* (Table 2). The lice exhibited a steady decrease both in numbers on the host and in numbers of infected birds as winter approached and then rose again with the onset of spring. On the other hand, the mite, *Cheyletiella,* was collected in midwinter and this parasite, in contrast to the feather-loving forms, lays its eggs directly on the surface of the skin. Thus its close association with the warm body may account for its occurrence during the cold weather. The blood-sucking mites, like the fleas, are associated not only with the host but also with its home, the nest, and consequently are far more abundant during spring and summer, the nesting period of the bird. Markov also noted that the endoparasites, except for *Isospora* and *Hymenolepis farciminosa,* decreased both qualitatively and quantitatively towards the end of the summer so that by October only these two parasites and *P. ensicaudatum* and *Capillaria ovopunctatum* persisted. This was not found to be the case in the present investigation for the helminthes, with the exception of the cestodes, remained relatively constant for both the 'summer' and 'winter' groups of starlings. There was a marked seasonal fluctuation as regards the degree of the tapeworm incidence (*H. farciminosa* and *Choanotaenia musculosa*) in that the number became reduced as winter approached, especially so when presence of whole worms was considered. Markov also observed a reduction of the tapeworm *C. musculosa* as the summer waned but noted its complete absence in October.

The rôle of *Sturnus vulgaris* as an agent in importing its parasites into North America is evident from the results of this investigation. The starling has certainly been the means of introducing the three lice, *Degeeriella nebulosa, Menacanthus spinosum, Myrsidea cucullaris;* the mite, *T. rosterii;* the tapeworms, *H. farciminosa* and *C. musculosa;* and the nematodes, *Capillaria ovopunctatum* and *C. exilis.* It has possibly brought in other parasites such as the flea, *Ceratophyllus gallinae,* the mites *Liponyssus sylviarum* and *Dermanyssus gallinae* (all blood-suckers), and among the endoparasites—*Isospora* sp., and the nematodes, *C. contorta, Dispharynx nasuta, Acuaria gracilis* var. *sturni* and *Porrocaecum ensicaudatum.*

As an outcome of these importations, some of these parasites have now been recovered from native birds. The possibility that the starling may be a disseminator of disease among poultry and cattle has been discussed by several investigators. Bullough (1943) suggested that it might be responsible for the unexplained outbreaks of foot and mouth disease in Britain as the sudden isolated attacks could be correlated with the distribution of the bird. Lewis (1926) believed that this bird is sometimes responsible for the distribution of gapeworm among poultry in Britain.

In North America this could not be so since so far, gapeworms have not been found in starlings. However, gallinaceous birds are known to be hosts for eight parasites recorded for *Sturnus vulgaris* in this country (Tables 1, 3). The list comprises the blood-sucking parasites—*C. gallinae, Haemaphysalis leporis-palustris, Ixodes brunneus, L. sylviarum* and *D. gallinae;* also two nematodes, *C. contorta* and *D. nasuta* and the acanthocephalan, *Plagiorhynchus formosus.* That host-specificity in flukes is slight is apparent when one surveys the host list given for four species of trematodes that have been recorded for the starling in Europe (Baylis, 1928; Sprehn, 1932) for this includes representatives from widely separated orders of birds—ANSERIFORMES, GALLIFORMES, COLUMBIFORMES and PASSERIFORMES. Under these circumstances it is to be exected that starlings may act as hosts to certain flukes that parasitize birds in this country. The MALLOPHAGA and the CESTODA exhibit marked host-specificity so that it would be impossible to find the same species of these groups of parasites in both the starling and gallinaceous birds. The starling may be relatively unimportant as a disease distributor among members of the GALLIFORMES. However, Clapham (1940) obtained evidence that English sparrows can carry eggs of helminths of poultry mechanically, either via the dirt present on their bodies or within their intestines where the eggs are passed unchanged from the gut. If this proves true in the case of the starling, then it must certainly be a menace to the well-being of poultry, as it so frequently inhabits chicken yards.

SUMMARY

1. A total of 300 starlings (153 juveniles and 147 adults) were examined for parasites (287 for ectoparasites; 300 for endoparasites). These were collected from six different states— Connecticut (6); Indiana (47); Massachusetts (41); Maryland (3); Ohio (19); and New York (184). The majority from New York—175 individuals including 149 juveniles and 26 adults, were killed in July, 1944, and between August and October, 1945. The rest of the birds were caught between November and April, 1944–45 and 1945–46.

2. All of the starlings examined were parasitized by one or more parasites; 95 per cent by ectoparasites and 99 per cent by endoparasites. Mites were represented in 67 per cent of individuals, and the lice, *Menacanthus spinosum* and *Degeeriella nebulosa* in 81 per cent and 72 per cent of cases. A percentage of 75 of birds contained *Isospora* sp.; 90 per cent were infected by helminths; 71 per cent by cestodes (56% by *Choanotaenia musculosa* and 34% by *Hymenolepis farciminosa*); and 68 per cent by nematodes (61% by intestinal *Capillaria*).

3. Seasonal fluctuations occurred for the MALLOPHAGA, the mite, *Trouessartia rosterii* and the starling's two tapeworms. The number of hosts infected by these parasites decreased as winter approached but increased with the onset of spring.

4. A total of 28 parasites has been found in the starling by the author (10 ectoparasites and 18 endoparasites). This investigation, and reports previously published, provide a total list of 34 parasites for this bird in North America (16 ectoparasites and 18 endoparasites).

5. In the present survey 15 first records for the starling in this country have been collected (indicated by asterisks in Tables 1 and 3). Of these, 9, as far as is known, are new records for *Sturnus vulgaris:* the three mites, *Dermanyssus prognephilus, Rivoltasia* sp. and *Cheyletiella* sp.; the unidentified flagellate; the three flukes,

Lutztrema sp., *Brachylaemus* sp. and *Leucochloridium certhiae;* and two nematodes, *Microtetrameres helix* (?) and *Acuaria gracilis* var. *sturni.* New state records have been established—*Degeeriella nebulosa* and *Menacanthus spinosum* for Connecticut, Indiana and Massachusetts; *D. prognephilus* for New York and *Plagiorhynchus formosus* for Ohio.

6. Original descriptions have been given for the eggs of *D. nebulosa, M. spinosum* and *Trouessartia rosterii,* and previous descriptions of *Choanotaenia musculosa* (hooks and egg), *Capillaria ovopunctatum* and *C. exilis* have been revised. The degree of penetration of some of the helminths into the wall of the digestive tract has been illustrated.

7. A general discussion is included regarding the starling as a source of material for a course in parasitology, as an importer of parasites into North America and as a disseminator of disease among native birds and poultry.

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RELATIVE AND SEASONAL ABUNDANCE OF THE COMMON RAT ECTOPARASITES OF SAN JUAN, PUERTO RICO

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Murine typhus fever in Puerto Rico is seasonal favoring the months of May, June, July and August when the number of cases reported almost always exceeds that of the remainder of the year. The disease is also urban and the majority of the cases are said to occur in the city of San Juan. An ectoparasite survey of the rats of San Juan, therefore, should show a prevalence of the vectors which is in accord with the seasonal variation of the disease and such arthropods ought to be among the most common ectoparasites of the city's rats. Of the many species of arthropods parasitic upon or associated with the rats of San Juan, six are so abundant as to merit special consideration. They are *Xenopsylla cheopis* (Rothschild), *Echidnophaga gallinacea* (Westwood), *Polyplax spinulosa* (Burmeister), *Bdellonyssus bacoti* (Hirst), *Laelaps nuttalli* Hirst and *Ornithodoros puertoricensis* Fox, of which the first four have for some years been known to be experimental transmitters of rodent typhus. What has been found out about the relative and seasonal abundance of these species in San Juan is given in the following pages.

The municipality of San Juan is located on a peninsula with San Juan Bay to the south and the Atlantic Ocean to the north. On the point of the peninsula is the old city or San Juan proper which except for a coastal suburb known as La Perla and an ancient military post, El Morro, contains commercial buildings and apartment-house type residences. The streets are paved with no empty lots, lawns or vegetation to speak of, and there is considerable activity out-of-doors day and night among the people. A short distance inland lies the district called Puerta de Tierra made up of various types of buildings, including residences, commercial establishments, warehouses and wharfs, with empty lots and some unpaved streets. On the base of the peninsula is Santurce, separated from Puerta de Tierra by a well-bridged estuary, it is mainly residential, the houses being for the most part small with front and back yards; empty lots, unpaved streets and vegetation are common, and in the district are varying socio-economic conditions which influence greatly the types of construction present.

As regards the places from which the rats came there was little ecological difference between the Santurce and Puetra de Tierra localities. The traps used were of the wooden box type baited with fresh bread. They were set in the backyards or under the houses (sometimes in them) of the poorer sections. The houses were usually small, frame structures, far from rat-proof and rats were reported to run in and out of them from the unpaved ground around them. The residents being not well-off economically were not very successful as a rule in maintaining the environs of their homes on a plane in accord with modern sanitary standards. In San Juan proper very different ecological factors obtained. Here unbaited steel snap traps

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TABLE 1.—*Number of rats captured each month in San Juan proper restaurants, April 25, 1946 through February 21, 1947*

Month	*R. norvegicus*	*R. rattus*
April	1	8
May	3	51
June	3	43
July	2	64
August	9	49
September	7	31
October	8	39
November	0	40
December	0	8
January	0	9
February	1	11
Total	34	353

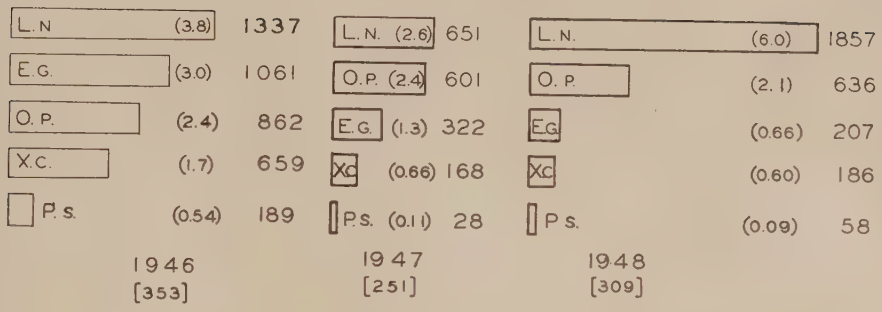
(Victor No. 0) were used and they were set inside restaurants and similar establishments in the business section. In both San Juan proper and Santurce two species of rats occur, *Rattus norvegicus* and *Rattus rattus* with its various color phases. But the ecological differences of the two districts have their reflection upon the rat population for in one, San Juan proper, the rats collected were about 90% *R. rattus* while in the other, Santurce, the rats collected were almost 90% *R. norvegicus*. (Tables 1 and 2).

At first it was planned to obtain comparable data from the San Juan proper business district and the Santurce residences over a period of several years. Before the end of a year, however, the San Juan proper survey had to be abandoned because of governmental rat-proofing activities as well as certain practical difficulties; but the Santurce survey went on for more than three years. The San Juan proper sur-

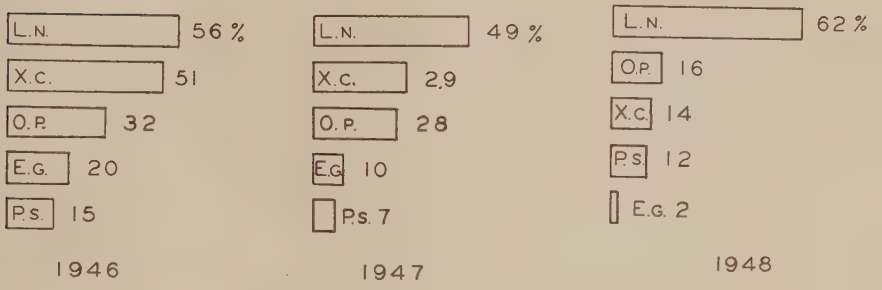
TABLE 2.—*Number of rats captured each month in Santurce, January 17, 1946 through December 31, 1948*

	1946		1947		1948	
	R. norvegicus	*R. rattus*	*R. norvegicus*	*R. rattus*	*R. norvegicus*	*R. rattus*
January	12	7	38	5	13	2
February	18	6	12	3	16	0
March	15	6	21	5	30	2
April	38	0	20	8	33	0
May	25	4	12	5	17	8
June	34	0	17	4	48	4
July	25	0	19	1	23	6
August	13	2	19	2	31	0
September	22	10	11	6	18	0
October	33	6	14	3	15	2
November	34	6	12	0	16	1
December	28	9	13	1	22	2
Totals	297	56	208	43	282	27

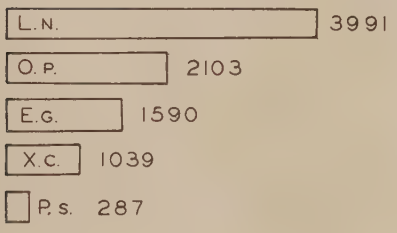
Fig. 1. A. Relative abundance of *Laelaps nuttalli, Echidnophaga gallinacea, Ornithodoros puertoricensis, Xenopsylla cheopis* and *Polyplax spinulosa* from rats of Santurce as shown by the mean (in parenthesis) and number of specimens collected during the years 1946, 1947 and 1948 based on the number of rats given below the years. B. Relative abundance of the same as shown by the infestation per cent. C. Relative abundance of the same as shown by the total number of specimens collected during the entire survey period of January 17, 1946 through March 8, 1949 based on a total of 938 rats. D. Relative abundance of the same as shown by the infestation per cent of each species for the entire survey period based on the total of 938 rats. E. Relative abundance of *Xenopsylla cheopis, Ornithodoros puertoricensis, Bdellonyssus bacoti, Laelaps nuttalli, Polyplax spinulosa* and *Echidnophaga gallinacea* as shown by the mean (in parenthesis), the number of specimens of each species collected and the infestation per cent based on 387 rats from San Juan proper restaurants taken during the period April 25, 1946 through February 21, 1947. F. Reported cases of murine typhus fever from Puerto Rico 1944 through 1948 as given by the U. S. Public Health Service in Public Health Reports.


L.N. (3.8) 1337
E.G. (3.0) 1061
O.P. (2.4) 862
X.C. (1.7) 659
P.s. (0.54) 189
1946
[353]
L.N. (2.6) 651
O.P. (2.4) 601
E.G. (1.3) 322
Xc (0.66) 168
P.s. (0.11) 28
1947
[251]
L.N. (6.0) 1857
O.P. (2.1) 636
E.G. (0.66) 207
Xc (0.60) 186
P.s. (0.09) 58
1948
[309]

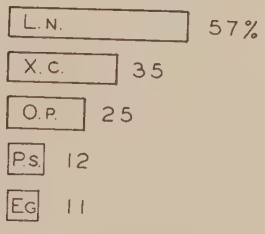
A


L.N. 56 %
X.C. 51
O.P. 32
E.G. 20
P.s. 15
1946
L.N. 49 %
X.C. 29
O.P. 28
E.G. 10
P.s. 7
1947
L.N. 62 %
O.P. 16
X.C. 14
P.s. 12
E.G. 2
1948

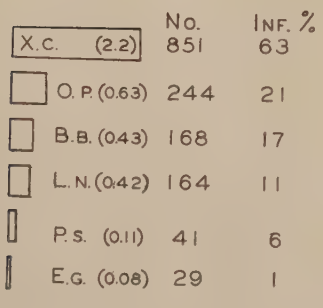
B


L.N. 3991
O.P. 2103
E.G. 1590
X.C. 1039
P.s. 287

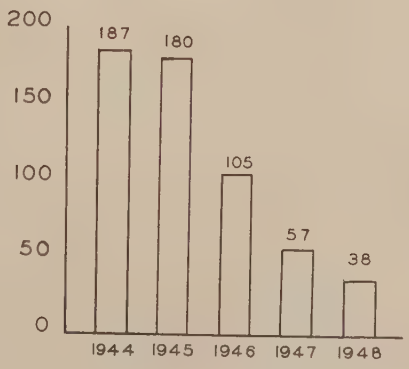
C


L.N. 57%
X.C. 35
O.P. 25
P.s. 12
EG 11

D


No. Inf. %
X.C. (2.2) 851 63
O.P. (0.63) 244 21
B.B. (0.43) 168 17
L.N. (0.42) 164 11
P.s. (0.11) 41 6
E.G. (0.08) 29 1

E


200
150
100
50
0
187
180
105
57
38
1944
1945
1946
1947
1948

F

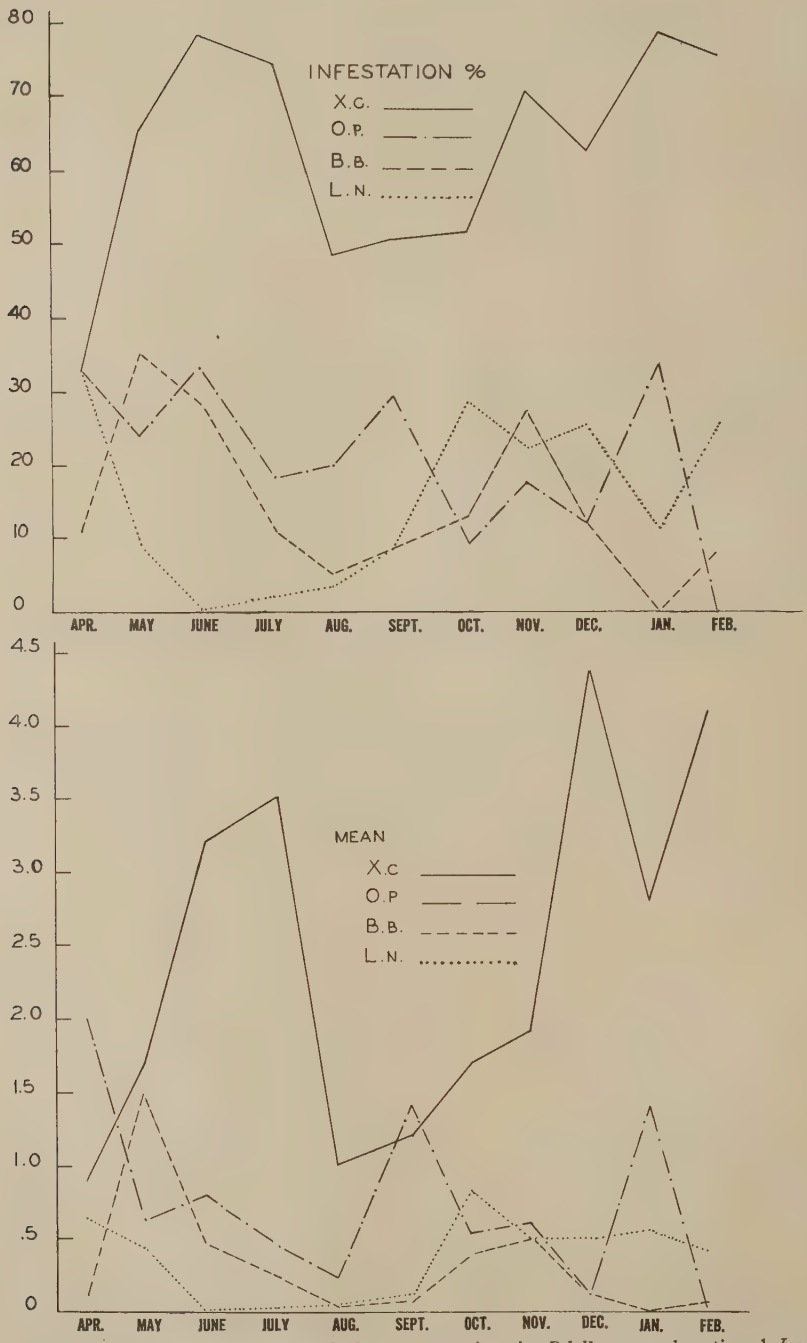


FIG. 2. *Xenopsylla cheopis, Ornithodoros puertoricensis, Bdellonyssus bacoti* and *Laelaps nuttalli* infestation per cent and mean by months from a survey of rats in restaurants of the San Juan proper business district, April 25, 1946 through February 21, 1947.

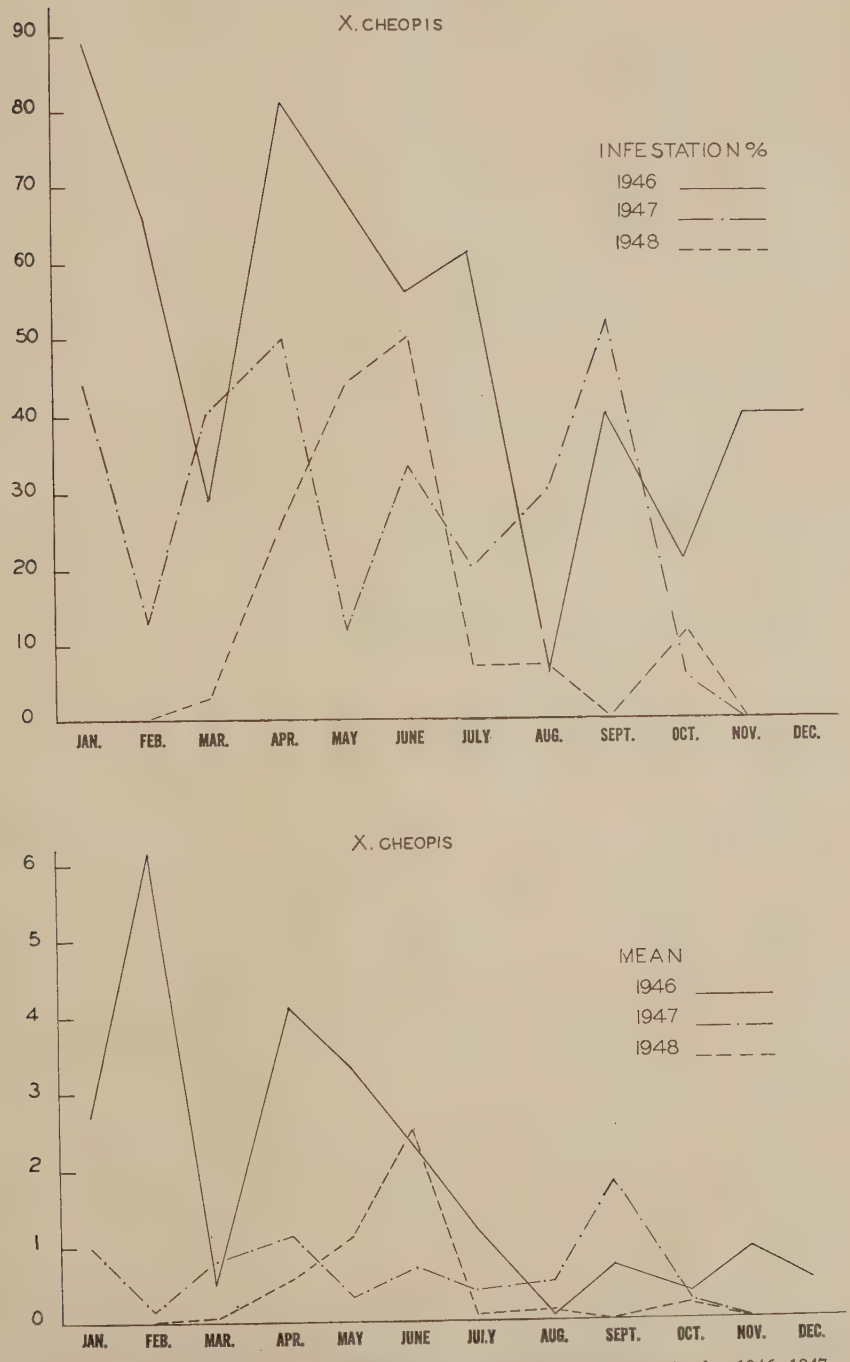


FIG. 3. *Xenopsylla cheopis.* Infestation per cent and mean by months for 1946, 1947 and 1948 from a survey of rats in or near residences in Santurce.

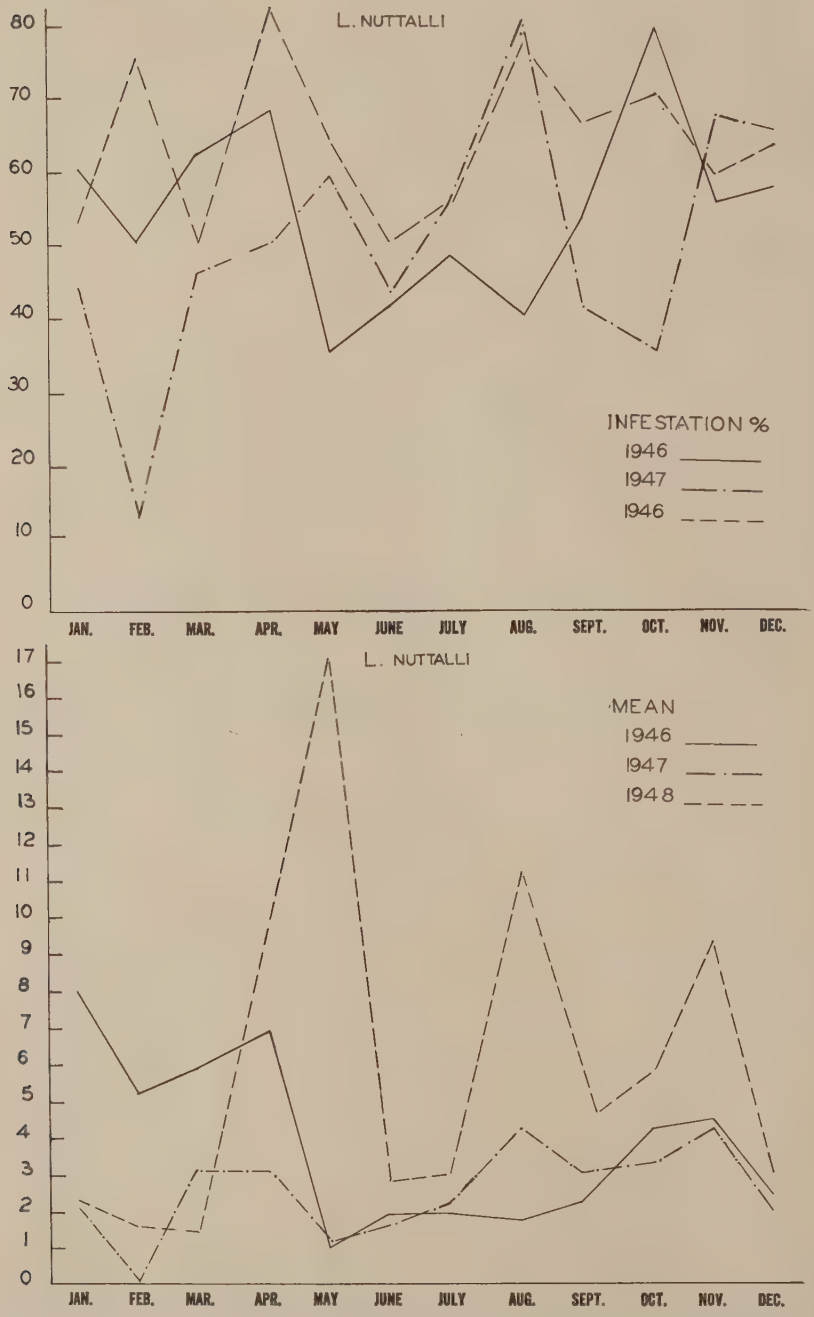


FIG. 4. *Laelaps nuttalli.* Infestation per cent and mean by months for 1946, 1947 and 1948 from a survey of rats in or near residences in Santurce.

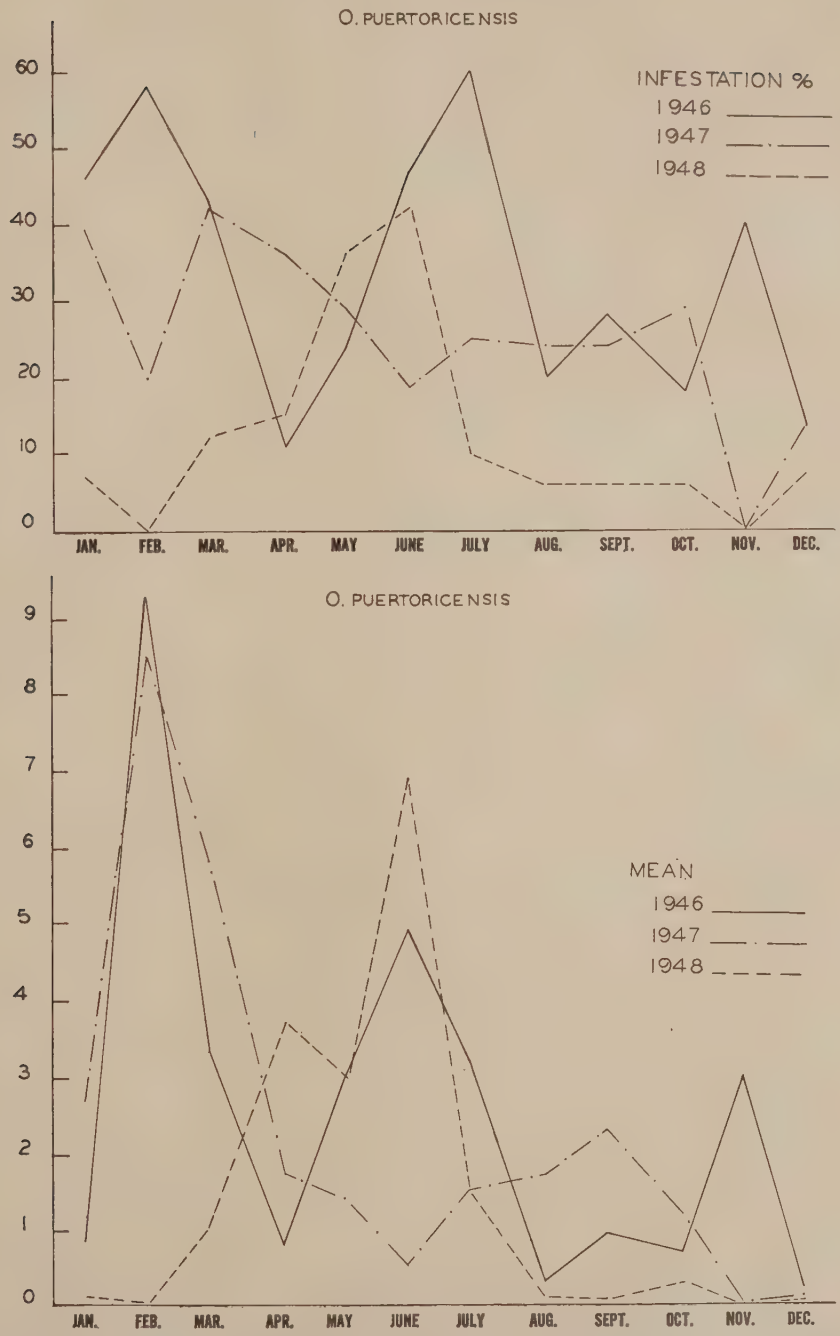


FIG. 5. *Ornithodoros puertoricensis.* Infestation per cent and mean by months for 1946, 1947 and 1948 from a survey of rats in or near residences in Santurce.

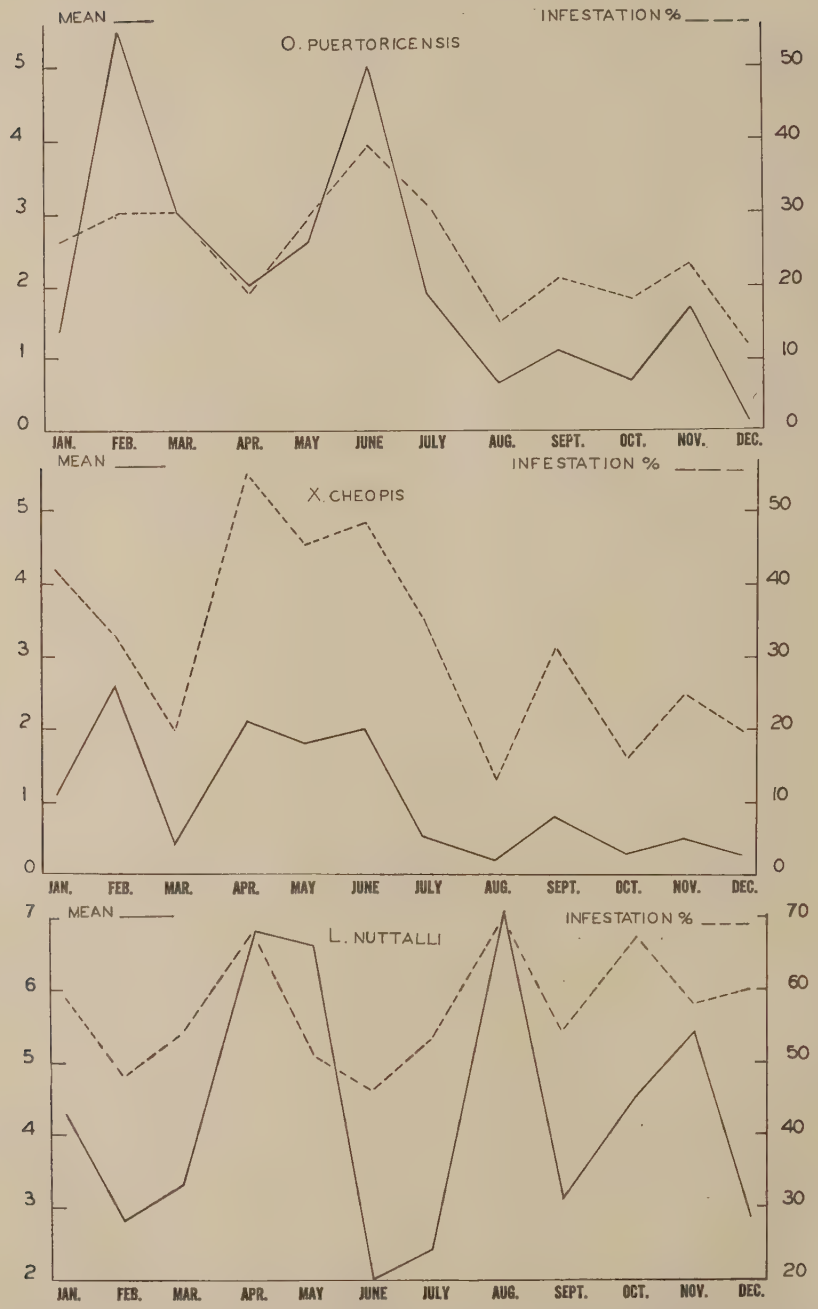


FIG. 6. *Ornithodoros puertoricensis, Laelaps nuttalli* and *Xenopsylla cheopis* mean and infestation per cent for 1946, 1947, 1948 and 1949 (January, February and March only), the data for the months being combined, from a survey of rats in or near residences in Santurce.

vey began on April 25, 1946 and ended February 21, 1947 after 387 live rats had been captured. The Santurce survey (within which are included some rats from Puerta de Tierra) began January 17, 1946 and ended March 8, 1949 after a total of 938 live rats had been obtained. The number of rats captured by calender month and species is given in Tables 1 and 2 below. (The data for 1949 from Santurce are as follows: January, *R. norvegicus,* 13, *R. rattus,* 2; February, *R. norvegicus,* 4, *R. rattus,* 1; March, *R. norvegicus,* 5; *R. rattus,* 0).

The relative abundance of the six common ectoparasite species based upon collections from 387 live rats captured in San Juan proper restaurants is shown graphically in Fig, 1, E. As regards the total number of specimens, the arithmetic mean (average number of specimens per rat) and infestation per cent (percentage of rats parasitized by a species) the order of abundance was the same with *X. cheopis* leading, 851 specimens having been collected giving a mean of 2.2 specimens; and 63% of the rats were infested with this parasite. Second in abundance was *O. puertoricensis* and thereafter came *B. bacoti, L. nuttalli, P. spinulosa* and *E. gallinacea,* with data as given in the figure. The mean and infestation per cent were calculated for each month and the results are presented in the graphs shown in Fig. 2. Neither in the mean nor in the infestation per cent did any species of ectoparasite show a seasonal distribution in accord with the reported cases of typhus in Puerto Rico. The most likely typhus vector suspect, *X. cheopis* showed peaks both in summer and winter months. In general the impression obtained from these graphs is that no definite seasonal occurrence can be attributed to any of the common ectoparasites infesting rats in San Juan proper restaurants. However, it must be pointed out that the number of rats collected each month was small and that the survey was not carried on for several years.

The Santurce survey of the poor residences was more complete in that it went on for several years. The same ectoparasites affecting the rats of the San Juan proper restaurants were found to infest the Santurce rats but there were noteworthy population changes. *B. bacoti* was not common, for only two rats were found infested in 1946, 11 in 1947 and three in 1948. The relative abundance of the other five species as regards the numbers of individuals collected and the means is shown in Fig. 1, A; and the relative abundance as given by infestation per cent is shown in Fig. 1, B. Both in numbers and infestation per cent *L. nuttalli* was the most common species. *X. cheopis* was consistently fourth as regards numbers but second or third as regards infestation per cent; and the place of the others (except for *P. spinulosa,* consistently the least abundant in numbers) varied somewhat from year to year. The total data for the entire period of the survey of 938 rats (including 25 taken in 1949) were analyzed with the results shown in Fig. 1, C. and D. It is apparent that *L. nuttalli* is the most abundant species. As regards the other species, the infestation per cents were not in accord with the numbers.

An interesting question occurred, could a relationship be found between the annually reported cases of typhus and the yearly population density of each ectoparasite? Figure 1, F. graphically shows the reported cases of typhus for Puerto Rico as a whole from the years 1944 through 1948 as given in Public Health Reports of the U. S. Public Health Service, from which figure it will be noted that the number of cases has decreased steadily and markedly. *L. nuttalli* was greater in abundance in 1948 than in the two previous years, hence it seems unlikely that it can be im-

portant as a vector, if it is true that vector abundance must be directly proportional to case abundance. *P. spinulosa* showed a steady reduction in mean from year to year but a great increase in infestation per cent in 1948 as compared with 1947. *O. puertoricensis* remained the same as regards mean in 1947 as in 1946, but was reduced in 1948; as regards infestation per cent, it decreased significantly each year. Only *X. cheopis* and *E. gallinacea* clearly were reduced both in mean and in the infestation per cent from year to year.

Neither *P. spinulosa* nor *E. gallinacea* were sufficiently abundant to permit a monthly analysis; but *L. nuttalli, X. cheopis* and *O. puertoricensis* were numerous enough to make a study worth-while. The monthly mean and infestation per cent for these three species was therefore calculated to ascertain if their seasonal distribution was in accord with the established fact that typhus is more prevalent in the summertime than at any other season of the year. The results are presented as graphs in Figs. 3, 4 and 5. From these graphs it will be immediately seen that in no case do the years confirm each other as regards the high points and low points. For the year 1948, however, *X. cheopis* and *O. puertoricensis* in both the mean and infestation per cent gave a distribution which corresponds to that of the disease.

Since relatively few rats were captured each month it is logical to believe that the localities from which the rats came would greatly affect the results, some places doubtlessly being more highly infested than others. In an attempt to eliminate geographical heterogenity an ideal year was created wherein the monthly data was pooled. The combined monthly results are shown as graphs in Fig. 6. It will be noted that the variation in abundance of *L. nuttalli* is definitely not related to the time of the year. But *O. puertoricensis* and *X. cheopis,* at least as regards infestation per cent, suggest a seasonal variation in accord with that of the reported cases of murine typhus.

SUMMARY AND CONCLUSIONS

1. In an effort to obtain knowledge of the relative and seasonal abundance of the common ectoparasites affecting the rats of the municipality of San Juan, it was found that ecological considerations including differences in the rat population necessitated two separate surveys: one, of the poorer districts of the residential section of the city known as Santurce and the other, of the restaurants in the business district of the portion of the city known an San Juan proper.

2. The results as regards relative abundance were very different in the two surveys. In San Juan proper the order of abundance was *X. cheopis, O. puertoricensis, B. bacoti, L nuttalli, P. spinulosa* and *E. gallinacea.* These data were obtained after about one year's work based on 387 rats. In Santurce results were obtained over a period of about three years which involved a total of 938 rats. They were consistent from year to year as regards *L. nuttalli,* which both in numbers and infestation per cent was the most abundant species. *X. cheopis* as regards numbers was consistently fourth in abundance; as regards infestation per cent it was second in 1946 and 1947 but third in 1948. *O. puertoricensis* as regards numbers was third in 1946 but second in 1947 and 1948; in infestation per cent it was third in 1946 and 1947 but second in 1948.

3. The monthly average number of specimens per rat and the monthly infestation per cent were calculated in an effort to ascertain whether there was a variation in abundance of ectoparasites related to the season of the year. The reason for this

is that the reported cases of typhus in Puerto Rico show an indisputable seasonal variation favoring the summertime. As regards San Juan proper variation in abundance of ectoparasites could not be related to the seasons of the year. It must be pointed out, however, that the San Juan proper survey was not carried on for several years. The Santurce survey was carried on for three years hence it was possible to obtain more complete data on seasonal abundance and also to compare the yearly results with the annually reported cases of typhus.

4. The following remarks apply to the Santurce survey only. On the basis that case prevalence is a function of vector prevalence, *L. nuttalli* must be eliminated from consideration as an important vector of human typhus because it was most abundant in a year of the lowest number of reported cases and because its abundance was not related to the seasons. One is inclined to eliminate *E. gallinacea, P. spinulosa* and *B. bacoti* also because they in general do not appear to be sufficiently numerous to account for the number of typhus cases. *X. cheopis* and *O. puertoricensis,* particularly the former, seem to meet the requirements for vectors inasmuch as they showed a drop in abundance from year to year corresponding with the drop in cases and they also suggest a seasonal variation which is in accord with the seasonal variation of the disease, especially if the infestation per cent is taken as a gauge of abundance.

ACKNOWLEDGMENTS

Thanks are due to the U.S. Public Health Service, District No. 6, for paying the salary of a rat trapper who caught most of the rats in the San Juan proper survey. Gratitude is also expressed to Dr. Américo Pomales Lebrón and Prof. José Rivera León of the School of Tropical Medicine, the former for facilities and advice and the latter for lettering Fig. 1.

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NOTES ON THE PHYSIOLOGY OF *MELOIDOGYNE JAVANICA* (TREUB, 1885), NEMATODA: HETERODERIDAE[1]

MARIE D. CHITWOOD

Though much work has been done on the physiology of nematodes parasitic in animals and considerable work is being done on the physiology of plants infected with parasitic nematodes, little basic work has been done on the physiology of nematodes parasitic in plants other than making direct observation on feeding or killing the organisms for control purposes. The following simple tests are offered with the idea that they show the need for study of phytoparasitic nematode physiology.

I. The nature of cell inclusions in *M. javanica* (= *Heterodera marioni*) and their significance.

The adult female, embryonated eggs and larvae of this species contain cell inclusions in the intestinal epithelium. These cell inclusions disappear as females become senile or larvae starve, if left active on a culture plate without the host plant.

A. One class of these inclusions consists of large clear droplets. They are insoluble in water, soluble in cold acetone, turn brown in osmic acid and run together at 53–54° but not at 52° C. They are liquid at 25° C., solid at 0° C. Their fusion with heat or soil fumigants is apparently a surface phenomenon, perhaps colloidal. When they run together the organism usually dies. This oil may be a neutral, slightly unsaturated ester as indicated by the facts that it reduces osmic acid and turns red in nile blue sulfate. Hoshina and Godfrey (1933) found a level place in their curve of lethal temperature durations of one of the root-knot nematodes. This level place occurred from 53–56° C. It would appear that coalescence of the oil droplets at this temperature indicates some colloidal change in the cells.

B. The second class of inclusions consists of smaller, solid colorless bodies. They are insoluble in water, acetone, etc., digested in pepsin and HCl and turn blue in triketohydrin hydrate. This indicates that they are protein, amino acids, peptides or peptones. Chitwood and Jacobs (1938) reported similar stored protein accumulating in the intestine of *Agamermis decaudata* during parasitic existence and disappearing during post-parasitic life when the organism is not feeding but is producing ova. The latter activity, of course, requires considerable protein. Root-knot nematodes also build up a protein reserve during the second to fourth stages of larval development and this material disappears during egg production. For those not familiar with the ninhydrin reaction, a broken specimen is placed in a small drop of distilled water. One or two crystals of triketohydrin hydrate are added. The specimen soaks in this solution 20–30 minutes and the water is drawn off as far as possible. A drop of vaseline is placed on the specimen, cover-slip added and slide heated. For permanent mounts the cover-slip may be removed from the slide after heating, vaseline removed by washing in xylene, and the specimen mounted in balsam. Single "granules" may be tested in this manner. However, the product of the reaction is

[1] This work done under the guidance of Dr. B. G. Chitwood, Dept. of Biol., Catholic Univ. America.

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water soluble and for identification of morphologic structures the water must be removed. Materials turning blue by this process contain a carboxy and an amino group.

II. The nature of the egg membranes.

Preliminary observations of the egg membranes of *M. hapla* and other nematodes were made by Chitwood (1938) and the vitelline membrane of *Ascaris lumbricoides* v. *suis* has recently been identified as myricyl palmitate by Timm (1950).

A. The egg shell proper. The egg shell proper has been identified as chitin by Krakow (1892) in *Ascaris,* by Chitwood (1938) in various nematodes including *M. hapla,* and by Jacobs and Jones (1939) in *Enterobius vermicularis.* In *M. javanica* it appears to be the same substance and quite permeable to most regents. Fauré-Fremiet (1913) and Szwejowska (1929) found glycogen diminution in eggs of *Parascaris equorum* during the formation of this layer, which is reasonable since chitin yields a glucosamine on hydrolysis.

B. The mucoid layer. This material, commonly called jelly, contains a protein (or glucoprotein) as is demonstrated by the ninhydrin reaction. When first deposited it is clear, colorless, and contains minute fibers. As it ages the outer surface becomes yellowish, then reddish brown. This color change gradually proceeds into the center of the jelly mass. As it proceeds the "jelly" becomes harder. The material can be decolorized by soaking in sulfurous acid (a reducing agent) and the reverse by fixing in formaldehyde or by prolonged exposure to air. This indicates a possible tanning reaction. Ellenby (1946) has previously reported polyphenol tanning in the cyst wall of *Heterodera rostochiensis* and Brown (1905) reports quinone tanning in the cuticle of various animals. The material in our case if "untanned" is incompletely digested by artificial gastric juice, Fairchild's trypsin, etc., and with prolonged exposure to dilute acids the eggs become free of the jelly and the solution without eggs gives a positive Molisch reaction indicating a sugar group. Since we have been unable to obtain the carbohydrate reaction in the absence of a ninhydrine reaction and vice versa, we feel justified in concluding the jelly contains a mucoid. As pointed out by Chitwood (1938) in *M. hapla* and Jacobs and Jones (1938) in *Enterobius vermicularis* this material is hygroscopic, tends to prevent drying of the eggs and to prevent entry of the egg by soil fumigants.

C. The vitelline membrane. This material is waxy, colorless with a melting point of 70.2° C. in *M. javanica.* It may or may not dissolve in acetone depending on temperature and concentration. When whole eggs were heated above 70° C. and the temperature dropped accidentally, this material crystallized within the eggs as burr-like birefringent crystals (individual crystals needle-like). A minute amount of U.S.P. beeswax was then dissolved by heating in acetone and on cooling the myricyl palmitate produced similar burr-like birefringent crystals. In the course of 24 hours, crystals of both origins lost their birefringency and became amorphous on a hot day (32° C.). An ethylene dibromide extract of *M. javanica* eggs, after washing quickly in cold acetone to remove the oils, was dissolved in hot acetone and yielded birefringent needle-like microscopic crystals on cooling. This material also lost its birefringency and became amorphous at 32° C. This substance has a melting point of 70–71° C. Vitelline membranes removed from eggs by cracking the eggshell are likewise soluble in warm acetone, reprecipitate in cold acetone in the form

of minute, needle-like birefringent crystals and melt at 70–71° C. The crystals in this case are not grouped in burrs but this is probably due to their being present in greater dilution than they are within the egg shell. The ethylene dibromide extract of whole eggs and the vitelline membrane behave in the same manner as does refined beeswax (myricyl palmitate).

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PARTHENOGENESIS IN THE ARGASID TICK *ORNITHODOROS MOUBATA* (MURRAY, 1877)

GORDON E. DAVIS*

Agamic reproduction in ticks was first reported by Aragão (1912) in a species of *Amblyomma* which he named *agamum* but which later was placed in synonymy with *A. rotundatum* C. L. Koch, 1844. He observed thousands of females which laid fertile eggs in the absence of males. The male of this species is still unknown. Nuttall (1913) observed the same phenomenon in *Rhipicephalus bursa.* Unmated females remained longer on the host than mated females, they did not become completely engorged, and the number of eggs produced was much smaller than in mated females. None of the larvae were reared to the adult stage. However, in later experiments with *R. bursa,* Nuttall (1915) considered that parthenogenesis was artificially induced by manipulation accompanying the enumeration of the eggs immersed in normal salt solution and rubbed gently with a camel's-hair brush. Bodkin (1918) reported parthenogenesis in *Amblyomma dissimile.* Sixty-five ticks of the first generation reared to adults were all females. Parthenogenesis was subsequently observed to the fourth generation. Brumpt (1934), Schulze (1937), and Floch and Abonnenc (1940) have expressed doubt as to the identity of the tick with which Bodkin worked. If the species of tick was *A. rotundatum* instead of *A. dissimile,* as has been suggested, the results are in complete agreement with the original report of Aragão for this species. The fact that the 65 ticks reared to adults in the first generation were exclusively females gives credence to their parthenogenetic origin.

Only one reference has been found to parthenogenesis in argasid ticks. Cunliffe (1921) reported that one of ten *O. moubata* females held for observation deposited 183 fertile eggs. However, he concluded that this female was probably fertilized unobserved and suggested that "further evidence is necessary before parthenogenesis can be considered to be even of rare occurrence in this species."

During a study of the quantitative transovarial transmission of *Borrelia duttonii* in *Ornithodoros moubata,* 600 ticks were reared from the first nymphal stage to adults by feeding individually at each nymphal stage and as adults on white mice. During the interfeeding periods, the ticks were stored individually in serially-numbered shell vials.

The 600 ticks were the progeny of three females, nos. 3, 6, and 9, and consisted of groups of 100 ticks each, following the first and second oviposition of each female.

In Table 1, it will be seen that of 100 ticks reared to adults following the first oviposition of ♀ 3, 46 were females, nine of which oviposited with none of the eggs hatching. Following the second oviposition, 53 of the 100 reared ticks were females, five of which oviposited with none of the eggs hatching.

Of the 100 ticks reared to adults following the first oviposition of ♀ 6, 48 were females with 23 ovipositing, five of which produced viable ova. Following the sec-

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ond oviposition, 60 of the 100 ticks were females, six of which oviposited with none of the eggs hatching.

The 100 ticks reared following the first oviposition of ♀ 9 resulted in 46 females, 33 of which oviposited, and 10 of which produced viable ova. The 100 reared following the second oviposition resulted in 37 females, 13 of which oviposited and two of which produced viable ova.

Eighty-nine of these 290 females oviposited but only 17 of them produced viable ova. In 11 instances, only one nymph emerged, in four instances 2, in one instance 3, and in one 6—a total of 28 agamic ticks of which 25 were reared to the adult stage. All were females.

Six females which had reproduced parthenogenetically were allowed to engorge a second time. None of them oviposited again over a period of 2 years.

In an additional series of 53 ticks reared in the same manner as the above 600, there were 27 females. Sixteen months after the adult feedings, 56 dead eggs, 16 first-stage nymphs, and four eggs which had hatched but the resultant larvae had not molted were found with one of the females. One of the four larvae molted 3 weeks later. Thirteen of these ticks were reared to adults. All were females.

TABLE 1.—*Summary—Parthenogenesis in* O. moubata

Female number and series	Number of females	Number of females ovipositing	Number of ticks which produced viable ova
♀3 F1S1	46	9	0
♀3 F1S2	53	5	0
♀6 F1S1	48	23	5
♀6 F1S2	60	6	0
♀9 F1S1	46	33	10
♀9 F1S2	37	13	2
	290	89	17

One parthenogenetic (F_1) female was allowed to mate with a normal male. There were 45 resultant eggs, all of which hatched, but 12 of the larvae died without molting. Twenty-eight of these were reared to adults. There were 15 males and 13 females.

DISCUSSION

When rearing *O. moubata en masse,* spermatophores are found attached to the females following the last nymphal molt and before they have had a blood meal. When feeding unmated females in the present study, the time and degree of engorgement did not appear to differ materially from mated females. However, oviposition was much delayed and the interval between hatching and molting was much prolonged. Fertilized eggs hatch and the larvae molt to the first nymphal stage almost simultaneously. When compared with the egg count in fertilized females there was a marked reduction in the number of eggs in the unfertilized females.

In contrast to the unfertilized eggs producing only females as reported by Bodkin for *Amblyomma dissimile* and the present findings for *O. moubata,* Skaliy and Hayes (1949) have recently reported that in another acarine parasite, *Liponyssus bacoti* (Hirst, 1913), unfertilized eggs develop into males which are capable of fertilizing normal females.

SUMMARY

Thirty-eight of 48 ticks hatched from unfertilized *O. moubata* eggs were reared to adults. All were females. Oviposition in unfertilized females was much delayed and the interval between hatching and molting of their progeny was much prolonged. When a parthenogenetic female was mated with a normal male both sexes were represented in the progeny.

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THE LABORATORY REARING OF THE COMMON FOWL TICK, *ARGAS PERSICUS* (OKEN)

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The utilization of ticks for teaching and research purposes is often precluded by the complexity of the life cycle as well as the time and space required for maintaining sufficient numbers of these blood-sucking arthropods. The laboratory rearing of ticks is ordinarily a rather long involved procedure, particularly when "hard ticks" (Ixodidae) are concerned. The Argaside, however, lend themselves somewhat more readily to colonization largely because of their shorter and less complex life cycle. Among the members of this group the fowl tick, *Argas persicus,* is the most widespread and the least difficult to obtain throughout its range.

This tick has almost a world-wide distribution in warm climates and is a vector of avian spirochetosis in many Old World regions (Cooley and Kohls, 1944). The disease has also been reported from Brazil, Panama and Cuba. In this country *Argas persicus* is distributed mainly through Texas, New Mexico, Arizona, Oklahoma, California and Florida according to Bishopp (1927). More recently, Cooley and Kohls (1944) and Bishopp and Trembley (1945) have listed additional records from Georgia, Louisiana, Nevada, Utah, Alabama, Mississippi and Canada. Reports on isolated infestations from certain other states have since been published. This tick has been associated with fowl spirochetosis in Texas by Brown and Cross (1941) and Burroughs (1947).

During a recent investigation of ticks in Texas it was desirable to keep a number of fowl ticks in the laboratory for a considerable length of time and to have the various growth stages readily available. A simple method devised for rearing this species is presented here.

MATERIALS AND METHODS

The ticks used to establish a colony were collected from a chicken roost in the vicinity of Grapevine, Texas. Initially, an attempt was made to duplicate as nearly as possible on a small scale the natural habitat of the tick. This was accomplished by nailing strips of wood to a circular wooden platform which was then placed on a layer of wood shavings in the bottom of a large metal can. With a millimeter or less space left between the wooden strips and the platform, a few adult ticks of each sex placed in the container will grow into a sizeable colony in a relatively short time with the necessary blood meals provided by placing a chicken in the can. Although this method was successful it had the disadvantage of rendering the ticks inaccessible since it was not feasible to remove and renail the strips of wood each time specimens were needed. A much more satisfactory method was subsequently developed (Figure 1).

Metal containers approximately $11\frac{1}{2}$ inches high and $7\frac{1}{2}$ inches in diameter were selected for this purpose. Four 2 inch diameter holes were punched in the tight-fitting lid. A piece of muslin was then glued on the top of the can keeping that part

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of the cloth over the holes free of glue. A strip of adhesive tape when wound tightly around the junction of the lid and the can was found to be quite adequate in preventing escape of the larvae. A layer of wood shavings about ½ inch deep was placed in the bottom of this container. A number of circular pieces of ½ inch Cellotex were cut with the diameter one inch less than that of the can. These were then cut in half in

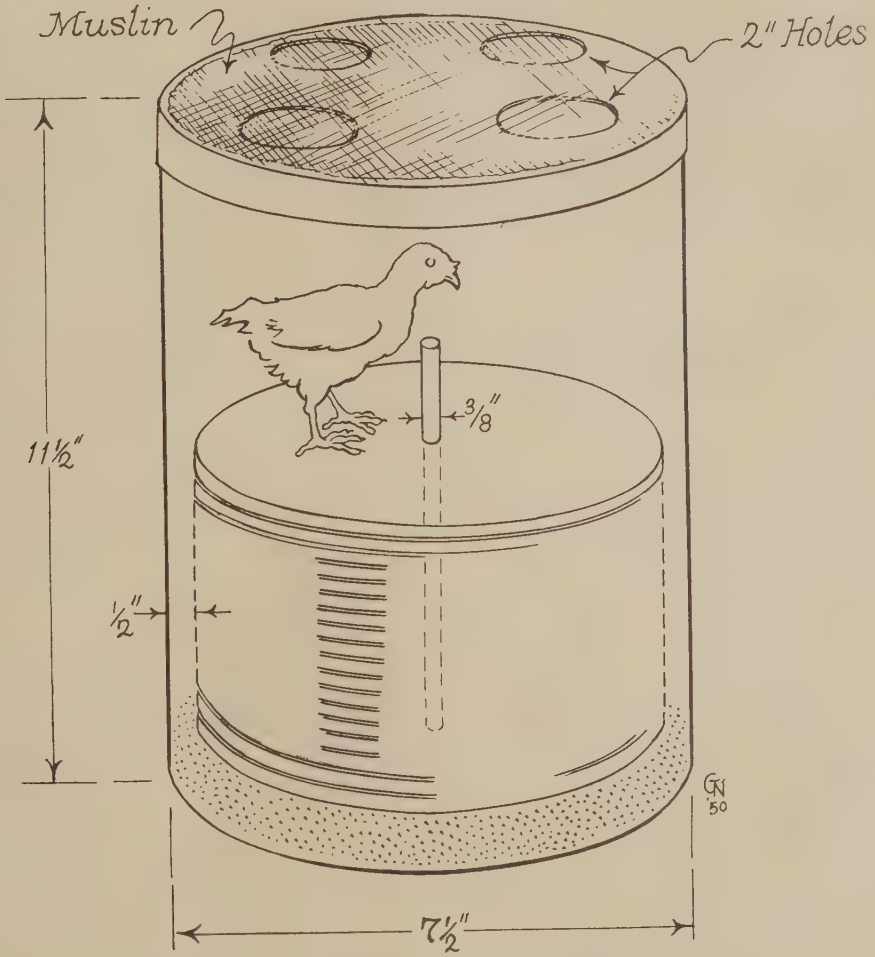

FIG. 1. Container for rearing and feeding ticks.

the horizontal plane with a dull instrument, leaving each half with a rough, irregular surface. The ticks were thereby provided with a better grasping surface during molting. A ⅜ inch diameter hole was bored through the center of the Cellotex discs and a wooden rod was inserted through these leaving one end extending above the uppermost disc to serve as a handle. A nail was driven through the lower end of the rod to prevent the lowermost disc from slipping off. When approximately 15 of these were arranged on the vertical rod so that they were almost touching one another and

with the lowest one resting on the wood shavings, there was ample space for a small chicken in the top of the can. Such an arrangement as this provided maximum cover for the ticks in a relatively small space.

The colony was fed by placing a young chicken in the can overnight or for several hours during the day. The four holes punched in the lid allowed sufficient ventilation and at the same time permitted daytime feeding of the arthropods by allowing very little light to enter. Unless very few ticks were present, the chicken was placed in the container at fairly frequent intervals or alternated with another to avoid death by exsanguination. This is particularly important when young chicks are used. When a considerably large number of larvae were seen on the uppermost disc or on the underside of the lid, a chicken was put in until all of them became attached. It was then removed and placed in a suitable small cage upon ascertaining that there were no nymphs or adults still feeding. When an infectious agent is used this cage must be surrounded by a trap of oil or other appropriate fluid to prevent escape of ticks.

With few exceptions, the larvae completed engorgement in 5 days. Therefore, on the afternoon of the fifth day following their attachment the chicken was transferred to a large beaker or other suitable container with wood shavings in the bottom and a heavy wire platform above that. The mouth of the container was covered with a piece of muslin and held in place by a rubber band. The following morning the engorged larvae, which were found at the top of the container, could be collected directly into a test tube. The remainder of the larvae were obtained by emptying the wood shavings into a white enameled pan and picked up with the aid of a camel's hair brush and forceps. If examination of the chicken revealed that all the engorged larvae did not drop off on the fifth night, the procedure was repeated on successive nights until the host was free of ticks.

OBSERVATIONS ON THE LIFE CYCLE

The tick colony was maintained at room temperature which varied from 25 to 28° C. and the relative humidity was between 70 and 80 per cent. Approximately 5 or 6 days after a complete blood meal, eggs were laid in batches averaging more than 100. The larvae hatched in 14 to 18 days and were ready for a blood meal shortly thereafter. Although they usually completed engorgement in 5 days, this period of time was extended at lower temperatures. The larvae lived as long as two months without a blood meal. Molting took place in approximately one week under laboratory conditions. Subsequent stages fed very rapidly, requiring from 15 minutes to two hours depending upon the stage involved. Each of the two nymphal stages lasted 12 to 14 days after which time adulthood was reached. The adults fed readily every 25 to 28 days, with a batch of eggs laid after each feeding. Both nymphs and adults fed largely on the legs and feet of the chicken, whereas the larvae attached themselves in areas where the skin is thin and readily penetrated by their mouthparts. One or more of the Cellotex discs were easily removed to obtain desired stages. Engorged females, when placed in stoppered vials or test tubes, laid batches of eggs very readily and all the progeny of one tick could thereby be isolated.

SUMMARY

1. A method of rearing *Argas persicus* in the laboratory is described, whereby a

relatively large number of ticks may be reared in a small space with a minimum of attention.

2. This method permits feeding of the ticks in the daytime as well as at night.

3. Detailed observations on the life cycle of the fowl tick under laboratory conditions are reported.

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A NEW CESTODE, *OOCHORISTICA PENNSYLVANICA*, AND SOME NEW OR RARE HELMINTH HOST RECORDS FROM PENNSYLVANIA MAMMALS

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A study has been made of a collection of helminths sent the senior author through the kindness of J. Kenneth Doutt of the Carnegie Museum. The specimens were collected by the Pennsylvania Mammal Survey, which is a cooperative feature between the Pennsylvania Game Commission, the U. S. Fish and Wildlife Service, and the Carnegie Museum. In the following notes the numbers in parentheses refer to collection numbers of the mammals in the Carnegie Museum.

In two specimens of *Blarina brevicauda* (1800, 3779) a species of *Oochoristica* was found. In No. 1800 the specimens were in such poor condition that no detailed study could be made, but as far as could be determined the worms in this host were similar to those in No. 3779. The latter constitute a new species for which the name *Oochoristica pennsylvanica* is proposed. Three complete worms, in fairly good condition, were present.

Oochoristica pennsylvanica n. sp.

Description: Strobila 58 to 97 mm. long, maximum width 1.9 to 2.2 mm. Scolex 636μ to 946μ across. Suckers large, 217μ to 233μ in diameter. Neck lacking; segmentation begins immediately behind scolex. Margin of worm serrate entire length. Width increases from 620μ to 700μ just behind scolex to its maximum of about 2 mm. at about one-third of its length. Segments broader than long throughout strobila. Mature segments 0.33 to 0.37 mm. long and 0.9 to 1.0 mm. wide. Gravid segments 0.5 mm. long and 1.9 to 2.2 mm. wide. Genital primordia first visible about 200μ to 300μ posterior to scolex. Genital atria irregularly alternating, muscular, about 45 to 48μ in depth, situated about 93 to 124μ from anterior border of proglottid. Three pairs of longitudinal excretory canals seen, but no transverse commissures observed. Genital ducts not distinguishable.

Testes 30 to 40, ovoidal, arranged in two lateral fields, not extending beyond lateral excretory duct. Cirrus pouch small, 78 to 93μ long, not reaching to lateral excretory duct.

Ovary large, reniform, in anterior median portion of segment. Maximum width in mature segments 232 to 279μ. Vitelline gland a compact mass 124 to 155μ in diameter, situated directly posterior to central part of ovary. Oötype and seminal receptacle not observed. Uterus spreads out rapidly over entire segment, and reproductive organs, except cirrus pouch, disappear. Onchospheres shrunken, not measurable.

Host: Blarina brevicauda.

Location: Small intestine.

Locality: Pennsylvania.

According to Hughes (1940), four other species of *Oochoristica* have been reported from insectivores, but none from the western hemisphere. No new ones seem to have been reported from insectivores since this review of the genus. *Oochoristica pennsylvanica* differs from *O. crinacii* Meggitt, 1920, from Asia and Africa, in being much longer and wider, with larger scolex and smaller cirrus pouch; from *O. herpestis* Kofend, 1917, from Sudan, in having a much narrower strobila, wider scolex and smaller cirrus pouch; from *O. incisa* Railliet, 1899, from France, by its broader strobila and scolex, much larger suckers, and smaller cirrus pouch; and

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from *O. parva* (von Janicki, 1904) Baer, 1935, from Cyprus, in having a much longer strobila, larger scolex, and fewer testes.

O. pennsylvanica more closely resembles *O. oklahomensis* Peery, 1939, from the spotted skunk, *Spilogale interrupta,* with respect to size of scolex, suckers and strobila, general shape, and measurements of the reproductive system, than any other species reported from mammals. It differs, however, in having the testes arranged in two lateral fields and in having the genital pores irregularly alternate instead of unilateral.

New or Rare Host Records

Catenotaenia pusilla. Several specimens were found in a *Pitymys pinetorum* (856). This worm has hitherto been reported from *Rattus norvegicus* in the United States (Leidy, 1855), but has also been reported from *Microtus arvalis, Evotomys glareolus* and other rodents in Europe. Our specimens agree with the description and figures of Joyeux and Baer (1945) except that some ripe proglottids reach a length of 4 mm. and have up to 16 or more main uterine branches on each side.

Macracanthorhynchus ingens. This acanthocephalan was found commonly in its usual host, *Procyon lotor,* but immature worms believed to belong to this species were also found in one *Mustela vison* (603), two *Mephitis nigra* (731, 733), one *Urocyon cinereoargenteus* (710), and in one *Parascalops breweri* (3804). The specimens, all in a severely contracted condition, reached a length of about 20 to 30 mm. in *Mephitis,* 10 to 20 mm. in *Mustela,* 8 mm. in *Urocyon,* and about 5 or 6 mm. in *Parascalops,* which is very little larger than the cystacanths freshly isolated from the intermediate host (Moore, 1946). There were four or five specimens in each skunk, three in the mink, one in the fox, and five in the mole. No details of structure could be clearly differentiated except the proboscis and proboscis sac. In at least one specimen from each host the proboscis was dissected out, split in two, and mounted in creosote in a spread-out condition so that the hooks could be accurately counted and measured. All agreed completely with similarly prepared proboscides of specimens from the usual host, *Procyon lotor.* They were also compared with the proboscides of other Acanthocephala possibly occurring in Pennsylvania which have somewhat similar hooks (*Macrancanthorhynchus hirudinaceus* and *Hamanniella tortuosa*), but were readily distinguishable. The occurrence of young specimens of this worm in carnivores other than the raccoon, and even in a mole, is not particularly remarkable since its relative, *M. hirudinaceus* of the pig, has been recorded from squirrels and chipmunk (Rausch, 1946). A number of immature oligacanthorhynchids have been reported from insectivores and other hosts in the Old World; their specific distinctness and relation to known adult worms is, according to Meyer (1933), uncertain.

Porrocaecum encapsulatum, described by Schwartz (1925) from under the skin of *Blarina brevicauda,* was found not only in several specimens of that host but also in a *Parascalops breweri* (3804), along with some *Porrocaecum americanum* (see below). The cysts containing the larval nematodes were found not only subcutaneously but also in the mesenteries.

Porrocaecum americanum, described by Schwartz (1925) from under the skin of *Scalopus aquaticus,* was found for the first time in *Blarina brevicauda* (3130) at-

tached to the outer wall of the small intestine, in *Sorex (fumaris?)* (3494) on the outer wall of the stomach, and in *Parascalops breweri* (3804), in the mesenteries. The cysts of this worm are very delicate and inconspicuous, so the worms appear merely imbedded in the stomach wall. One cyst contained three worms, but all the others had single worms. These nematodes agree with Schwartz's description in all respects except the presence of two small hyaline tooth-like processes on either side of the mouth, one conical with rounded tip, the other shorter and flat-topped. Although Schwartz believed both his species to be distinct from *"Ascaris incisa"* and *"Ascaris acanthura"* encysted in the viscera of European insectivores, it seems possible that *P. americanum* may be identical with *Ascaris incisa* Rudolphi, which Leuckart thought might be the larva of *Porrocaecum depressum,* a species found commonly in hawks and owls in both Europe and North America.

Ascaris laevis. Several male and female specimens of this species, briefly described by Leidy (1856) from a single specimen in *Marmota monax,* and not recorded since, were found in one specimen of *Marmota monax* (2108). These specimens along with some obtained by Robert Rausch from Alaskan ground squirrels, are to be described in a separate publication by Dr. Jack D. Tiner.

Strongyloides sp. A single female of this genus was found in a *Procyon lotor* (230). This is the first report of *Strongyloides* in a raccoon, but *S. nasua* was reported by Darling (1911) from the related *Nasua narica* in Panama.

Trichostrongylus calcaratus. This common rabbit parasite was found in a muskrat, *Ondatra zibethicus.* It was reported from this host once before, by Barker (1915), under the name *T. fiberius.* This is more fully discussed in a separate publication (Chandler, 1950b).

Rictularia onychomis. Several female specimens from a *Peromyscus leucopus* agree in all details with Cuckler's (1939) description of this species except that the teeth bordering the stoma number 20 instead of 26.

Gnathostoma spinigerum. This species, better known from southern Asia, has been reported from North American mink on a few occasions only (Erickson, 1946). Single specimens were found in each of two mink, *Mustela vison* (741, 747). A careful comparison with specimens of *G. spinigerum* from Calcutta failed to reveal any specific differences.

Gongylonema pulchrum. This worm was found in abundance in the tongue of *Euarctos americanus* (218). A report of the finding and a discussion of the possible synonymy of *G. pulchrum* with *G. ursi* (Rudolphi, 1819) has been published (Chandler, 1950a).

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